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***5086 volunteer abstracts, 18 symposium and workshop abstracts, 4 special lecture abstracts.**

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# CHRONOLOGICAL LIST OF SESSIONS

**CONTINUED FROM *SOCIETY FOR NEUROSCIENCE ABSTRACTS*, VOLUME 11, PART 1**

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# THEMATIC LIST OF SESSIONS

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| | | |
|---|---|---|
| 1986 | November 9—14 | Washington, DC |
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219.11 CHANGES IN NEURONAL FUNCTION PRODUCED IN CAT PRIMARY SOMATOSENSORY CORTEX BY THE IONTOPHORETIC APPLICATION OF ACETYLCHOLINE. R. Metherate*, N. Tremblay* and R.W. Dykes. Depts. Physiology, Neurology and Neurosurgery, and Surgery, McGill University, Montreal, Quebec, Canada.

It is known that acetylcholine (Ach) increases the firing rate of some neurons in cat primary somatosensory cortex. However, little is known regarding the influence of Ach on neuronal responsiveness to afferent inputs. Because Ach has also been correlated with long term changes in membrane resistance (Woody et al, 1978), experiments were designed to search for changes in responsiveness over extended time periods. Cats were anesthetized with sodium pentobarbital, a craniotomy performed over somatosensory cortex and stimulating electrodes placed in the ventroposterior thalamus. Individual barrels of 5-barrel micropipettes were filled with a carbon fiber, Ach (1.0M), glutamate (0.5M), atropine (25mM) and sodium chloride (3M). Single neurons were recorded though the barrel containing the carbon fiber and subjected to various combinations of the drugs delivered iontophoretically. During data collection, somatic stimuli and/or pulses of glutamate were applied at regular intervals before, during, and after Ach delivery. Lesions were placed at two levels in penetrations of interest and, after the experiment, the electrode trajectory was reconstructed from the histological section containing the electrode track.

301 neurons were studied in 45 cats. As expected Ach increased firing rates in some cells, (17.2%) and decreased it in others (2.3%). Atropine blocked the increased firing due to Ach. Ach either enhanced or diminished the responsiveness of a cell to tactile stimuli or to iontophoretically-applied glutamate (46%; 27/59). Generally, these alterations disappeared and the cell responses returned to control values within 5 min.

In contrast, when Ach was delivered simultaneously with glutamate or when Ach was delivered during strong stimulation of the receptive field, altered responsiveness occurred in 80% (43/54). It often lasted for more than 7 min and sometimes more than 1 hr.

These observations suggest that 1) long-term or plastic changes can occur in individual somatosensory cortical neurons, 2) Ach may be a mediator of these changes, and 3) that the changes also depend upon factors related to neural activity of the modified neuron. Such a mechanism may underlie the reorganization of cortex following digit amputation (Merzenich et al, 1983) while is accompanied by local increases in Ach receptors (Sampson et al, 1984). (Supported by NIH, FRSQ and MRC).

219.12 GABAERGIC INHIBITION IN THE SECOND SOMATIC SENSORY AREA OF CATS. K.D. Alloway and H. Burton. Dept. of Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

Many interneurons in somatosensory cortex are believed to be GABAergic. Previous reports have indicated that blocking GABAergic receptors in somatosensory area I increases the receptive field size of rapidly-adapting neurons but does not alter their adaptive properties (Hicks and Dykes, 1983). We have commenced a series of experiments aimed at determining the role of GABA in shaping the dynamic properties of neurons in the second somatic sensory cortical area (SII).

Cats, previously implanted with a chamber over SII, were anesthetized with ketamine and acepromazine, placed in a sling apparatus, and non-traumatically immobilized. Following isolation of single neuron activity, ketamine was discontinued and the animal was artificially respired and paralyzed with flaxedil. Acepromazine continued to be administered to provide tranquilization. Single units were recorded with a platinum wire in the central barrel of a 9-barrel glass pipette. Side barrels contained 5-6 mM bicuculline methiodide (BMI), 0.25 M GABA, 0.3 M glutamate, and 1.0 M sodium chloride. All electrode penetrations were made in the part of SII representing the glabrous skin of the central pad. Vibrotactile stimulation of the central pad was used to activate isolated SII neurons.

At this time, results have been obtained from a sample of 12 SII neurons. All cells responded to iontophoretic applications of glutamate with an increase in spontaneous activity that was dose-dependent; thresholds for activation ranged from 10 to 40 nA. By contrast, GABA produced a dose-dependent inhibition of both spontaneous (when present) and stimulus-evoked activity; current levels needed to produce at least a 50% decrease in stimulus-induced activity were variable and ranged from 5 nA (2 neurons) to 100 nA, with a mean of about 35 nA for all 12 neurons.

Two neurons have been studied throughout exposure and recovery from BMI. For both cells 10 nA ejected sufficient quantities of BMI to block the inhibitory effects of GABA on stimulus-induced activity. This dose of BMI caused only a moderate increase in spontaneous activity, but notably increased response entrainment to sinusoidal cutaneous vibration. Thus, both cells responded with greater periodicity to a 20 Hz stimulus following BMI and often exhibited a burst of 2 or 3 spikes in the same portion of the stimulus cycle. Several additional cells responded to BMI with an increase in stimulus-evoked activity but were not tested with sinusoidal stimulation. These preliminary results suggest that a GABAergic inhibitory circuit may limit the temporal fidelity of cortical responses to vibrotactile stimuli.

Supported by NINCDS grants NS09809 and 5T32 NS07071.

219.13 CORTICAL CONNECTIONS OF AREAS 2, 1, AND 5 OF SOMATOSENSORY CORTEX IN MACAQUE MONKEYS. T. P. Pons and J. H. Kaas. Department of Psychology, Vanderbilt University, Nashville, TN 37240.

The cortical connections of electrophysiologically defined parts of the body representations in somatosensory cortex of 13 macaque monkeys, (12 Macaca mulatta, 1 M. fascicularis) were determined after separate or combined injections of wheat germ agglutinin (WGA) conjugated with horseradish peroxidase, tritiated WGA or tritiated proline. After extensive microelectrode mapping of a portion of the body representations in areas 3b, 1, 2, and 5 and careful determination of electrophysiological borders between areas, small restricted injections of tracers were made usually into the hand or foot representations in areas 1 and 2. All cortical connections were reciprocal; however, there were differences in the laminar patterns of connections between areas.

Following injections of tracers into the hand or foot representations in area 2 label was consistently observed in areas 3a, 3b, 1, 4, 5, 7, and in the S-II region. The densest label following area 2 injections was in the S-II region and the sparsest label was in area 4. Separate foci of label were usually found rostrally and caudally in area 4. In one case with an injection into the foot representation, additional label was observed in the region of the supplementary motor area. Following injections of tracers into the area 1 hand and foot representations, cortical label was observed in areas 4, 3a, 3b, 2, 5, 7, and the S-II region. Again the densest connections were with the S-II region. In one case with an injection into the foot representation, additional label was observed in the region of the supplementary sensory area. After an injection in the representation of the forearm in area 1, dense label was observed in the part of area 5 that has been shown to contain a cutaneous representation of the forearm and wrist in anesthetized animals (Pons et al., '85, Brain Res.). In a single case in which the forearm representation in the highly cutaneous zone of area 5 was injected with tracer, dense label was observed in the portion of area 1 representing the arm and forearm. Additional label was seen in areas 4, 7, and the S-II region. A lateral portion of area 5 was also labeled. Feedforward laminar patterns with terminal label concentrated in layer IV, and feedback patterns with label concentrated in nongranular layers suggest that the processing of cortical information proceeds from area 3b to both areas 1 and 2. Areas 1 and 2 then relay information to areas 5 and 7 and to the S-II region. Each of these areas in turn feed back onto areas earlier in the processing sequence. Thus, the cortical processing of somatosensory information has both serial and parallel components. Funded by NIH Grant NS16446.

219.14 THE REPRESENTATION OF THE FACE, ORAL CAVITY, AND TEETH IN AREA 3b OF SQUIRREL MONKEYS, SAIMIRI SCIUREUS. C. G. Cusick and J. T. Wall. Dept. of Psychology, Vanderbilt University, Nashville, TN 37240.

In order to study the topographic organization of mechanoreceptor inputs from the face and oral cavity in somatosensory cortex of monkeys, fine grained microelectrode mapping experiments were performed in squirrel monkeys with up to 389 recording sites within the face region of the dorsolateral surface of the brain. Separate representations of the face were were identified in areas 3b and 1, and the representation in area 3b was determined in detail.

Within area 3b, cortex related to the chin was just lateral to that devoted to the hand. More caudally, area 3b was activated from the lateral face. Caudomedially, the area 3b-1 border represented skin on the midline of the upper head with the scalp, supraorbital skin, nose, and midline of the upper lip, forming a mediolateral cortical sequence. More rostrally, the area 3b-1 border represented the lateral lower lip, followed by the teeth and oral structures. Along the rostromedial border of area 3a with 3b, the somatotopic organization varied. The pattern in 3 monkeys closely resemblesd the organization in S-I of non-primates and prosimians in that the midline of the lower lip projected to the 3a-3b border, which represented the chin, lower lip and lower teeth in caudorostral sequence. In two cases, the upper lip was represented at the 3a-3b border, between the chin and teeth, and lower lip was displaced from the border. The upper lip was either continuously represented from the area 3a-3b to the area 3b-1 border, or the representation was split by the lateral lower lip.

The representation of the teeth was explored up to 83 recording sites in a single experiment. The representation in area 3b was roughly topographic and surprisingly large, ( 4 $mm^2$). Receptive fields varied in size, with fields on incisors including fewer teeth than fields on the molars. Receptive fields for molars usually included both upper and lower teeth. In general, the lower incisors were represented caudally, adjacent to the representation of the lower lip, and the molars were located more rostrally. The upper incisors were usually along the 3a-3b border. In different cases, the upper incisors were either adjacent to the upper lip, the chin, or the lower lip caudally, or to the palate rostrally. The variability of the representation of the lips and teeth in area 3b in squirrel monkeys differs markedly from the relatively consistent representations of the hand in these monkeys. Supported by NIH Grant DE06554.

219.15 THE SOMATOTOPIC ORGANIZATION AND CONNECTIONS OF A THIRD AREA OF SOMATOSENSORY CORTEX IN RODENTS. L. A. Krubitzer, M. A. Sesma, and J. H. Kaas, Dept. of Psychology, Vanderbilt University, Nashville, TN 37240.

In contrast to carnivores and primates where a number of cortical representations of the body surface have been found, only two somatosensory representations, the primary (S-I) and secondary (S-II) somatosensory areas, have been reported in rodents. In the present study, anatomical and electrophysiological methods were combined to determine the organization and connections of somatosensory cortex in grey squirrels, Sciurus carolinensis. Grey squirrels offer the advantages of larger brain size and generally more distinct architectonic subdivisions than do common laboratory rodents. Microelectrode mapping methods were used to define subdivisions of somatosensory cortex, and microlesions were placed to mark physiological borders. Then, wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP) was injected into physiologically identified sites within S-I, S-II, or into a newly identified representation, the parietal ventral area (PV). The cerebral hemispheres were flattened, and alternate sections were reacted for WGA-HRP, or stained for myelin.

The results revealed reciprocal connections between S-I and the following fields: (a) S-II; (b) motor cortex; (c) the parietal ventral area (PV) caudal to S-I and ventral to S-II; (d) the parietal medial (PM) area caudal to S-I and medial to S-II; and (e) the parietal rhinal area (PR) ventral to PV and just dorsal to the rhinal sulcus. Like S-I, S-II had reciprocal connections with motor cortex, PV, PM, and PR.

Injections in PV confirmed connections with S-I and S-II, and revealed additional connections with motor cortex, PM, and PR. Callosal connections were with PV, PR, S-I, and S-II. Recordings in PV revealed neurons responsive to light tactile stimulation of skin and hairs. Receptive field sizes for neurons in PV were larger than those for S-I but similar to those for S-II. PV was somatotopically organized as an inverted "homunculus" with the limbs directly medially, the trunk ventrally, and the face congruent with the representation of the upper lip and nose in S-I. At least part of PV was also activated by auditory clicks.

The results indicate that grey squirrels have at least three somatotopically organized cortical representations of the body surface, and potentially as many as five somatosensory fields. One of these fields, PV, appears to be bimodal.

Supported by NIH Grant NS16446.

219.16 LOCALIZATION OF HUMAN SENSORIMOTOR CORTEX IN SURGERY BY RECORDING OF SOMATOSENSORY EVOKED POTENTIALS. T. Allison, C.C. Wood, D.D. Spencer*, P.D. Williamson*, W.R. Goff* and G. McCarthy. VA Medical Center, West Haven, CT 06516 and Yale Univ. Sch. of Med., New Haven, CT 06520.

The traditional means of localizing sensorimotor cortex involves mapping sensory and motor responses elicited by electrical stimulation of the cortical surface. This procedure accurately localizes sensorimotor cortex but is time consuming and is best carried out in an awake, cooperative patient. We have developed cortical surface somatosensory evoked potential (SEP) recordings as a technique for localizing sensorimotor cortex in patients operated under general as well as local anesthesia. Using a 64-electrode grid placed in the vicinity of the hand representation area of sensorimotor cortex, SEPs to contralateral median nerve stimulation were recorded. In 24 patients the validity of SEP localization was verified by independent localization by cortical stimulation; in all cases the two methods were in agreement. In 17 patients the hand area was successfully localized under general anesthesia by SEP recording without cortical stimulation. Three criteria are useful for localization; (1) Polarity inversion of N20-P30 potentials to P20-N30 potentials as electrode locations cross the central sulcus from the postcentral to the precentral gyrus; these potentials are thought to be generated in area 3b of somatosensory cortex. (2) P25-N35 potentials are largest in a small region of postcentral gyrus about 1 cm medial to the largest N20-P30; these potentials are thought to be generated primarily in area 1 of somatosensory cortex. (3) Regardless of specific component identification, large SEPs are recorded only from sensorimotor cortex.

219.17 ANATOMICAL DETERMINATION OF THE EXTENT OF OVERLAP IN THE SENSORIMOTOR REGION OF THE TAMMAR WALLABY, *MACROPUS EUGENII*.

L. Mayner. Department of Behavioural Biology, R.S.B.S. Australian National University, Canberra, A.C.T. 2601, Australia.

Physiological and anatomical studies have shown conclusively that in the American opossum, *Didelphis virginiana*, the motor and somatosensory neocortical regions are totally overlapped. Anatomical studies on Australian marsupials, the possum, *Trichosurus vulpecula*, and native cat, *Dasyurus viverrinus*, have shown that there is only partial overlap between the motor and somatosensory neocortical regions. However, the physiologically defined neocortical motor area of the possum was found to be far more extensive than that determined anatomically. A physiological study on the Australian wallaby (Lende, R. *Science*, 141: 730, 1963) showed that there was total overlap between the motor and somatosensory neocortical areas in this Australian marsupial. The aim of this study was to determine the extent of overlap between motor and somatosensory regions in the neocortex of the wallaby. The sensorimotor area occupies the whole of the parietal neocortical region in the brain of the wallaby. The sensorimotor region was found to contain two prominent layers, layers IV and V, throughout the entire extent of the region. Following HRP injections into the physiologically defined body subdivision of the sensorimotor area label was located in the ventrolateral nucleus, VL, as well as the ventral posterolateral nucleus, VPL. HRP injections into the rostral head subdivision of the sensorimotor region gave rise to label in VL as well as the ventral posteromedial nucleus, VPM. However, HRP injections into the caudal head subdivision of the sensorimotor region gave rise to label only in VPM. The caudal head subdivision was found physiologically to represent the upper lip and vibrissal face regions. Thus, there is extensive, but not total, overlap between the motor and somatosensory neocortical areas in the wallaby. Since layer V is prominent in the caudal head subdivision of the sensorimotor region this could suggest that the physiologically defined motor region is more extensive than the VL projection field. Thus, the VL projection field is not delineating the entire extent of the motor region, this could be a feature inherent to Australian marsupials which appear to exhibit only partial overlap between the motor and somatosensory regions.

219.18 **INTERRELATIONSHIPS BETWEEN CYTOCHROME OXIDASE, GABA AND METABOLIC (2DG) LABELING IN PRIMATE SI CORTEX.**

**Sharon L. Juliano, Oleg Favorov* and Mark Tommerdahl*. Dep Physiology, University of N. Carolina, Chapel Hill, NC 27514.**

**In the visual system of primates, specific cerebral cortical locations (known as "blobs") preferentially take up stain for the mitochondrial enzyme cytochrome oxidase (CO). These blobs of cells possess discrete functional characteristics and exhibit precise positional relationships to cells which stain for GABA. Also, depending on the stimulus conditions, these blobs of cells either overlie or interdigitate with patches of cortex which are active during 2-deoxyglucose (2DG) experiments. We have initiated studies in the primate somatosensory cortex (SI) which explore the relationships between CO, 2DG and/or GABA. In these studies, macaca fascicularis monkeys underwent a 2DG experiment in which a tactile stimulus was delivered. Following the 2DG experiment, sequential sections were processed for 2DG, stained histochemically for CO and stained immunohistochemically for an antibody directed against GABA. As in earlier studies, the labeling produced by 2DG was clearly patch-like. Although less distinctly patchy, the labeling obtained with CO was not homogeneous but showed clear and consistent fluctuations in the density of uptake. A computer image analysis system allowed us to digitize both the 2DG and CO sections and to generate two- or three-dimensional maps of each form of labeling. When the 2DG and the CO labeling obtained from adjacent sections were compared either as individual sections or as unfolded cortical maps, a distinct relationship between the two forms of label was seen. The two patterns of activity were reciprocally complimentary for the stimulus conditions used in these 2DG experiments (brush strokes to the hand or arm). In other words, regions of cortex exhibiting elevated 2DG activity displayed low mitochondrial enzyme (CO) activity while cortical regions of low 2DG uptake displayed high concentrations of CO. The neural elements which stained heavily for GABA demonstrated no absolute relationship to either the 2DG or CO labeling. However there was a tendancy for the cortical regions which stained heavily for GABA to be coincident with regions of increased 2DG uptake, while regions of SI cortex which stained poorly for GABA tended to be coincident with regions of increased CO activity. Although the functional significance of these relationships needs further investigation, the regions of SI cortex which contain high CO activity may represent functional zones which are excited by stimulus conditions different from those used during the 2DG experiments.**

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219.19 MORPHOLOGY AND LAMINAR DISTRIBUTION OF NEURONS WITH GLUTAMATE-LIKE IMMUNOREACTIVITY IN THE RAT SOMATOSENSORY CORTEX. F. Conti*, A. Rustioni, P. Petrusz*. Department of Anatomy, University of North Carolina, Chapel Hill, N.C. 27514.

Electrophysiological, biochemical and autoradiographic studies strongly suggest a neurotransmitter function for Glutamate (Glu) and/or Aspartate (Asp) in the mammalian cerebral neocortex. The present study was aimed at determining the morphology and the laminar distribution of the neurons that contain Glu in the rat somatosensory cortex using an immunocytochemical technique. For this purpose, an approach similar to that described by Ottersen and Storm-Mathisen (J. Comp. Neurol., 1984) was used.

An antiserum was raised in rabbits against glutamate conjugated to hemocyanin by glutaraldehyde. The characterization of this antiserum has been described elsewhere (Hepler et al. J. Histochem. Cytochem, 1985). Adult, male Sprague-Dawley rats were perfused with normal saline followed by a fixative containing either 4% paraformaldehyde and 0.3% glutaraldehyde or 5% glutaraldehyde in phosphate buffer. Free-floating, 25 μm thick vibratome sections and 7 μm thick paraffin sections were stained using the double PAP technique.

In all cases so far studied about 30% of neurons in the somatosensory cortex were immunoreactive. These were present in all layers, although in different proportions. Immunoreactive neurons were least numerous in layers I and IV (6.6% and 18%, respectively) and most numerous in layers V and III (38% and 35%, respectively). The proportion of positive neurons was 32% in layer II and 30% in layer VI. In all layers, except layer I, most of the immunoreactive neurons were pyramidal in shape, although non-pyramidal positive neurons were also observed.

The present results are in good agreement with those of previous studies in showing that a significant number of somatosensory cortical neurons probably use Glu and/or Asp as neurotransmitter. Furthermore, the laminar distribution as well as the morphology of stained neurons support the view that Glu and/or Asp are neurotransmitters in the descending corticofugal and in the associative and callosal cortico-cortical pathways. This hypothesis can now be tested by combining the immunocytochemical technique used in the present study with retrograde labeling of the neurones projecting to the different targets of the somatosensory cortex.

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219.20 PHYSIOLOGICAL AND PHARMACOLOGICAL STUDIES ON NEURONAL FUNCTION IN THE RAT CEREBRAL CORTEX IN VITRO. N. Hori,* I. Hirotsu*, T. Ishihara,* N. Katsuda* and D.O. Carpenter (SPON: J. Ramaley). Dept. of Pharmacology, Faculty of Dentistry, Kyushu Univ., Fukuoka 812, Japan, Lab. of Experimental Pharmacology, Suntory Inst. for Biomedical Research, Mishima-gun Osaka, 618 Japan and Wadsworth Center for Labs & Res., NY State Dept. of Health, Albany, NY 12201.

The physiologic and pharmacologic properties of the neuronal network within the neocortical columns was examined in an in vitro rat somatosensory cortex preparation. Rectangular brain slabs (0.6 X 0.6 X 2.3 mm) were cut from the somatosensory cortex perpendicular to the pial surface. Procedures used for incubation and recording of electrical activities were similar to those used for thin brain slices (Hori et al., 1982). Electrical stimuli (negative 1-10 V, 0.05 msec) were applied to the white matter by a single electrode and evoked antidromic spikes and orthodromic responses in all the layers of the cortex (I-IV). The width of functional neuronal columns was 250 to 300 μm as indicated by the pattern of excitation observed upon shifting the recording or stimulating electrodes laterally.

In normal Ringer solution stimulation of the white matter evoked a single antidromic spike and/or orthodromic spike in layers II and IV. Intracellular recordings show various responses including an antidromic spike followed by an EPSP or IPSP, no antidromic spike but an EPSP with superimposed spikes, etc. When the slice was perfused with a solution containing d-tubocurarine ($10^{-4}$ M) or bicuculline ($10^{-5}$ M), a well synchronized repetitive train of spikes (interval, about 5 msec) was observed and these trains, except for a short latency antidromic spike, were completely blocked in low $Ca^{2+}$ and high $Mg^{2+}$ solutions. Similar repetitive spikes were obtained in atropine ($10^{-4}$ M), but the train showed less synchronization and was of shorter duration. Intracellular recordings showed that atropine ($10^{-4}$ M) depolarized the membrane by about 10 mV. These results suggest that at least two kinds of interneurons may be involved in the control of the pyramidal neuron. One of those is an excitatory interneuron which may have inhibitory nicotinic receptors and the other is an inhibitory interneuron which may release GABA and have excitatory muscarinic receptors. If these two interneurons receive the same recurrent projections from the pyramidal cells (Lorente de No, 1938), curare and bicuculline may induce a synchronized repetitive train of spikes. Atropine also may inhibit the activity of the inhibitory GABA neuron, and then the pyramidal cell becomes more excitable.

219.PO VIBROTACTILE STIMULATION AS A TOOL FOR CORTICAL MAPPING IN MAN WITH POSITRON EMISSION TOMOGRAPHY. H. Burton, P.T. Fox*, M.E. Raichle. Depts. Anatomy & Neurobiology, Neurology & Neurological Surgery, and Radiology, Washington U. Sch. Med., St. Louis, MO 63110.

Regional cortical responses to vibratory stimuli were recorded in 24 normal, human volunteers using positron emission tomographic (PET) measurements of regional cerebral blood flow (rCBF). An intravenous bolus of $^{15}O$-labeled water was administered as a blood flow tracer (J. Nucl. Med. 24:782). Each subject underwent a series of 8 sequential, 40-sec, CBF emission scans with an inter-scan delay of 8-10 min (for isotope decay). No stimulus was given during the initial or final scans in a series. Stimuli were applied during the intervening 6 scans, with stimulus onset preceding scan initiation by 40-60 sec. All subjects underwent large amplitude stimulation (LA) of the fingers (D1-5) of one or both hands (2 mm amplitude; 130 Hz; 5 cm tip). One protocol (n=9) included: LA stimulation of the halux; lip, left & right D1-5 and bilateral, alternating (2 Hz), flexion-extension finger movements (FM). Highly-discrete, small amplitude stimulation (SA) of individual digit pads was also tested across a range of frequencies (20-300 Hz sinusoids; <500 μm amplitude; 2 mm tip; n=5). All responses were computed as % change in rCBF from resting-state value (J. Neurophysiol. 51:1109). Anatomical localization within PET images was determined stereotactically relative to the AC-PC line (J. Comput. Assist. Tomogr. 9:141).

Intense, focal increases in rCBF occurred in every individual in the cerebral hemisphere contralateral to LA finger stimulation (mean 31%). No significant cerebral responses were detected ipsilateral to any unilateral stimulus. Handedness did not affect response magnitude or location. The post-central gyrus (SI) was the response locus for LA and SA, as determined from the mean stereotactic coordinates of the areas of maximal rCBF change. Response locale systematically varied with the body part stimulated, distributing across cortex in a medial-to-lateral/superior-to-inferior sequence from lower limb (halux), to upper limb (D1-5), to face. Moreover, D1 (thumb) responses lay lateral to and separated from those of D5 (SA). Antero-posterior separation was obtained between the loci of peak response to FM (anterior) and LA, although considerable response overlap occurred partly due to the magnitude of LA and FM responses and to limited spatial resolution (13 mm in plane). Application of different stimulus frequencies (20-300 Hz; SA) to the same digit did not affect response localization.

The consistency and magnitude of LA responses make this tool well-suited for clinical PET research, e.g., to test perfusion reserve in cerebrovascular disease. SA stimulation, however, is preferable for PET studies of somatosensory cortical localization, as this stimulus is discrete and well-characterized.

219.PO PROJECTION PATTERNS FROM THE VENTRO-BASAL COMPLEX OF THE THALAMUS TO THE RAT BARREL CORTEX. S. M. Lu* AND C.-S. Lin; DEPARTMENT OF ANATOMY, DUKE UNIVERSITY, DURHAM, N. C. 27710

Previous electrophysiological studies of layer IV neurons in the posteromedial barrel subfield (PMBSF) of primary somatosensory cortex (SI) have suggested that a large portion of barrel neurons, especially those residing in the sides of barrels, respond to more than one contralateral mystacial whisker (Simons & Woolsey, '79). It is possible that such barrel neurons receive converging input from thalamic axons of the ventro-basal complex (VB) representing different whiskers. The goal of the present study was to determine whether single VB axons innervate more than one barrel.

A mixture of 10% HRP (Boehringer Grade I) and 2% WGA-HRP (Sigma) was iontophoretically injected into the VB for 20 minutes using 4 uA of positive current. The anterogradely HRP filled axons and their terminals were then visualized with the DAB-GOD method (Itoh et al., '79).

The branching of VB axons may occur at different levels, ranging from the white matter to upper layer V. Some VB axons have a branch that innervates the center of one barrel, and another branch that innervates the side of an adjacent barrel. Others innervate only one barrel.

In addition, both large and small diameter axons arborize in layer I. Some of the large axons also send collaterals to layers VI, V, IV and II/III.

The terminal fields of VB axons form patches which are located mainly in the centers of the barrels in layer IV. Heaviest VB terminals are found in layer IV and decrease gradually to layers II/III. Less dense terminal fields are found in layers I and VI.

In conclusion, we have found evidence that individual VB axons may innervate more than one barrel. This may be an anatomical basis for the finding that barrel neurons respond to more than one whisker. (Supported by NS 06233 to C. S. Lin).

220.1 PHARMACOLOGICAL PROPERTIES OF OPIATE-INHIBITED ADENYLATE CYCLASE IN RAT STRIATAL MEMBRANES. S.R. CHILDERS, P. NADEAU AND P. NIJSSEN. Department of Pharmacology, University of Florida College of Medicine, Gainesville, FL 32610.

A well established second messenger system coupled to opiate receptors is inhibition of adenylate cyclase. Previous studies (Childers and LaRiviere, J. Neurosci. 4, 2764-2771, 1984) have shown that the small (10-20%) inhibition of adenylate cyclase by opiates in brain membranes can be increased to 30-40% maximal inhibition by preincubation of membranes at pH 4.5, which alters function of GTP-coupling (N) proteins, decreasing function of the stimulatory unit $N_s$ while increasing function of the inhibitory unit $N_i$ in brain membranes. We have used this technique to quantitate the pharmacological properties of opiate-inhibited adenylate cyclase in rat striatal membranes. All experiments were conducted in buffer containing 100 mM NaCl and 50 μM GTP. Under these conditions, opioid peptides were good agonists, inhibiting adenylate cyclase by 30-40% with $IC_{50}$ values between 0.2 and 0.8 μM for enkephalin, delta receptor enkaphalin analogs (DSLET, DADLE), beta-endorphin, and several dynorphin analogs. Morphine and mu-receptor enkephalin analogs (morphiceptin, FK-33824) were weaker, with $IC_{50}$ values betwen 5 and 20 μM, and were often not full agonists, with maximal inhibitions of 20-25%. Naloxone, levallorphan, and naltrexone were equipotent in blocking inhibition by all agonists studied; however, the delta antagonist ICI-174864 was ineffective in antagonizing opiate inhibition. Irreversible blockade of [$^3$H]-opiate binding sites by 80% with naloxone azine, p-nitro-phenyl-naloxone or p-nitro-phenyl-oxymorphone did not affect either basal or opiate-inhibited adenylate cyclase. These results suggest that receptors coupled to opiate-inhibited adenylate cyclase in striatal membranes may not correspond to any of the classical opiate receptor types determined in receptor binding studies.

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220.2 [$^3$H](+)SKF10,047 BINDING IN RAT AND MOUSE BRAIN. R. Sircar and S.R. Zukin. Depts. of Psychiatry and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

Martin in 1976 postulated the existence of the sigma class of opioid receptors based on the pharmacology of the drug N-allyl-normetazocine (SKF10,047). While the ability of this agent to precipitate withdrawal in morphine-dependent animals stems from its potent mu-antagonist actions, its stimulant and psychotomimetic properties cannot be explained on the basis of interactions with the classical opioid receptor types. Increasing behavioral and biochemical evidence supports the concept that the unique effects of SKF10,047 and those of phencyclidine (PCP) and its derivatives are mediated via a common sigma opioid/PCP receptor. The (+) sigma opioids, devoid of activity at classical opioid receptors, thus seemed promising as "pure" molecular probes of sigma opioid/PCP receptors. In this study the binding characteristics of [$^3$H](+)SKF10,047 (25.5Ci/mmol) in rat and mouse brain homogenates were determined in the presence and absence of excess nonradiolabeled (+)SKF10,047 by a rapid filtration assay (4°C, 5mM Tris-HCl, pH 7.4). Scatchard analysis revealed a curvilinear plot in both species. Computer-assisted, non-linear regression analysis indicated a statistically valid fit of the data to a two-site but not to a one- site model. In order to determine the identities of the sites, displacement studies with opioids, PCP derivatives, dioxalans and neuroleptics were carried out. Haloperidol and pentazocine were potent displacers of [$^3$H](+)SKF10,047 from the high-affinity site; potencies of drugs at this site did not correlate with their potencies for eliciting PCP-, SKF-like or other known behaviors. The potencies of drugs at the low-affinity sites agreed with the rank orders previously determined for binding to PCP/sigma opioid receptors. In at least these two species [$^3$H](+)SKF10,047 thus interacts with two classes of binding sites: the sigma opioid/PCP receptor and a higher-affinity site whose behavioral significance is at present unknown.

220.3 NALTREXONE-INDUCED SUPERSENSITIVITY: FUNCTIONAL CORRELATES IN THE MOUSE AND RAT. B.C. Yoburn, G.S.F. Ling, G.W. Pasternak and C.E. Inturrisi*, Depts. of Pharmacology and Neurology, Cornell Univ. Med. Coll. and Lab of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center, New York, NY 10021.

Long-term exposure (8 day) to the opiate antagonist naltrexone (NTX) produces an approximately 50% increase in opioid binding sites in rat brain and increases morphine's analgesic potency by 1.5 times compared to placebo-treated rats (Yoburn et al., Life Sci., 1985, in press). Rats exposed to NTX for 24 hrs show no significant changes in binding or analgesia. In an effort to see if NTX-induced alterations extended to another opiate effect and another species, we examined the effects of NTX exposure on respiratory depression in rats and morphine analgesia in mice.

Mice were implanted with a single NTX (8 mg) (N=13) or placebo pellet (N=15) for 8 days. The pellets were removed and 24 hrs later baseline nociception was determined using the tailflick procedure. Groups did not differ ($p>.05$) in baseline flick latencies. Mice were injected with 1.5, 3.0 or 6.0 mg/kg morphine, s.c., and tested for analgesia at 30 and 60 min. Mice that did not flick by 10 sec in either test were defined as analgesic. Significantly more mice pretreated with NTX were analgesic (62%) than placebo-treated (20%) mice ($p<.05$).

In the second experiment, rats were implanted with two, 35 mg NTX (N=6) or placebo (N=6) pellets for 8 days. The pellets were removed, arterial and venous catheters inserted and 24 hrs later baseline blood samples were taken and rats injected with 3 mg/kg morphine, i.v. Blood samples were drawn periodically for 90 min and measured for pH, $pO_2$ and $pCO_2$. There were no significant differences ($p>.05$) prior to morphine (pH = 7.54, 7.52, $pCO_2$ = 40.20, 41.37, $pO_2$ = 90.14, 87.89; for NTX- and placebo-treated, respectively). Following morphine, equivalent respiratory depression was observed for both groups ($p>.05$). Peak mean responses at 7.5 min post-drug were: pH = 7.47, 7.44, $pCO_2$ = 47.03, 46.83, $pO_2$ = 69.42, 76.83; for NTX and placebo-treated, respectively.

These studies extend the phenomenon of NTX-induced supersensitivity to morphine analgesia to another species; the mouse. In the rat, NTX treatment does not produce supersensitivity to morphine-induced respiratory depression. This finding may be related to a selective functional upregulation of receptors regulating analgesia ($\mu_1$) but not receptors mediating opiate-induced respiratory depression ($\mu_2$) in the rat. (Supported in part by NIDA Grants DA-01457 and DA-02615.)

220.4 METAPHIT, AN IRREVERSIBLE INHIBITOR OF PHENCYCLIDINE (PCP) RECEPTORS, ACYLATES A PORTION OF [$^3$H]3-PPP BINDING SITES IN RAT BRAIN. L. L. Coughenour*, W. G. Berghoff*, M. F. Rafferty* and T. A. Pugsley*. (SPON: J. Woods). Warner-Lambert/Parke-Davis, Pharmaceutical Research, Ann Arbor, MI 48105.

Metaphit (1-[1-(3-isothiocyanatophenyl)cyclohexyl]piperidine), a derivative of PCP, specifically acylates about 50% of the binding sites in rat brain striatal and hippocampal membranes labeled by [$^3$H]-PCP (Rafferty et al., FEBS 181:318, 1985). The benzomorphan or sigma opiates and PCP and its derivatives bind to both [$^3$H]PCP labeled sites and [$^3$H]benzomorphan labeled sites in a strikingly similar manner (Quirion et al., PNAS, 9 79:5881, 1981; Zukin and Zukin, Mol. Pharmacol. 20:246, 1981) suggesting that the psychomimetic effects of both the benzomorphan opiates and PCP may be mediated through a common receptor. Recently, a non-opiate, (+)[$^3$H]3-(3-hydroxyphenyl)-N-n-(1-propyl)-piperidine ((+)[$^3$H]3-PPP), has been reported to bind with high affinity to the sigma opiate receptor in rat brain (Largent et al., PNAS, 81:4983, 1984). There are some discrepancies between [$^3$H]-PCP labeled binding sites and radiolabeled sigma opiate binding sites, notably that the potent dopaminergic antagonist haloperidol inhibits (+)[$^3$H]3-PPP binding and the binding of the benzomorphan opiate [$^3$H]-SKF-10047 with nanomolar affinity and does not have high affinity for [$^3$H]-PCP binding (Largent, ibid; Tam, PNAS, 81:5618, 1984). If PCP, (+)3-PPP and the sigma opiates are binding to the same site, metaphit would be expected to acylate the sigma opiate receptor as well as the PCP labeled receptor. We have investigated the effects of metaphit on the binding of (+)[$^3$H]3-PPP to the sigma opiate receptor in rat brain membranes. Acylation of whole rat brain membranes (Rafferty et al.) and (+)[$^3$H]3-PPP binding assays (Largent et al.) were carried out as described. Metaphit acylated 40% of the binding sites labeled by (+)3[$^3$H]3-PPP (Control Bmax: 25 pmoles/mg tissues, Kd: 60 nM; Metaphit treated Bmax: 15.3 pmoles/mg tissue*, Kd: 58 nM) without a change in affinity. Thus, Metaphit appeared to acylate (+)[$^3$H]3-PPP binding sites in a manner similar to [$^3$H]PCP binding sites. In control membranes PCP inhibited the binding of (+)[$^3$H]3-PPP ($IC_{50}$ = 2.4 μM) in a distinctly biphasic manner (slope 0.64), but in metaphit treated membranes the affinity of PCP was decreased 5 fold, and the slope of the inhibition curve approached unity. In rat brain (+)[$^3$H]3-PPP appears to label two sites; one of which binds (+)3-PPP and PCP with high affinity and is sensitive to acylation by Metaphit. *$p<$ 0.005, Student's t test; N = 3 separate experiments done in triplicate.

220.5 NALTREXONE INDUCES DIFFERENTIAL UP-REGULATION OF RAT FOREBRAIN MICROSOMAL AND SYNAPTIC MEMBRANE μ OPIOID RECEPTORS. A.M. Moudy, J.W. Spain* and C.J. Coscia (SPON: K. Smith). Departments of Biochemistry and Physiology, St. Louis University Sch. Med., St. Louis, MO 63104.

Chronic administration of the opiate antagonist, naltrexone, to rats has been shown to result in up-regulation of their brain opioid receptors. Using subcellular fractionation techniques we have resolved brain opioid receptors into two membrane populations, one associated with synaptic plasma membranes (spm) and the other enriched in smooth endoplasmic reticulum and Golgi (microsomes) (Roth et al., J. Biol. Chem. 256, 10117, 1981). In this study we addressed the question of whether naltrexone induces up-regulation uniformly in these two populations. Rats were administered naltrexone by subcutaneously implanted osmotic minipumps which delivered 5 mg/kg/day of this long-lasting antagonist for 7 days. Thereupon, forebrain μ opioid receptor levels were determined by homologous displacement of [$^3$H]D-ala$^2$-mePhe$^4$-gly-ol$^5$ enkephalin followed by estimation of binding parameters using the LIGAND program.

Receptor levels in crude membranes rose 77% after treatment. Microsomes displayed a 92% increase in μ receptor concentration, representing an almost two-fold greater change than that associated with spm fractions (51%). This difference was statistically significant ($p<0.01$). Neither changes in binding affinities ($K_d$ = 1.4 nM) nor membrane protein content were detected. These results establish that naltrexone induces up-regulation of both microsomes and spms. Furthermore, they are consistent with the hypothesis that microsomal and spm opioid receptors represent discrete populations of intracellular and cell surface sites, respectively. Supported by NSF grant BNS 81-14947 and NIH grant HL 07050.

220.6 REGIONAL STUDIES OF SUFENTANYL INHIBITION OF $^3$H-NALOXONE BINDING IN THE RAT BRAIN BY QUANTITATIVE AUTORADIOGRAPHY. W.A. Geary, II and G.F. Wooten. Dept. of Neurology, Univ. of Virginia, Charlottesville, VA 22908.

We have examined regional competition curves of the mu-selective agonist sufentanyl (Janssen, Belguim) against 3.0 nM $^3$H-naloxone binding( 51 Ci/mmol, Amersham) to determine regional densities of mu binding in intact rat brain tissue. $^3$H-naloxone was used as a nonselective probe for multiple opioid binding subtypes (James and Goldstein, Mol. Pharmacol. 25:337-342, 1984); sufentanyl (0.25-12.5 nM) was used to selectively demonstrate regional mu binding activity by competitive inhibition (James and Goldstein, ibid, 1984). Quantitative autoradiographic procedures were employed to measure binding to in vitro incubated 20 μm cryostat sections of rat brain; quadruplicate serial sections were prepared every 200 μm in the coronal plane from the level of the olfactory bulb through the locus ceruleus (roughly 250 sections per rat); $n \geq 2$ rats for each sufentanyl concentration studied. All experiments were performed in phosphate buffered saline; quantitative analysis was carried out as previously described (Geary and Wooten, J. Pharm. Exp. Therap. 225:234-240, 1983). All data were corrected for differential autoabsorption of tritium (Geary and Wooten, Brain Res. 1985, in press).

Twenty-four brain regions were analyzed for sufentanyl competition for $^3$H-naloxone binding; regions were chosen based on previous reports of variable densities of opioid binding subtypes. Sufentanyl displaced $^3$H-naloxone binding monophasically in all regions examined. In addition, the percent inhibition across all brain regions was highly uniform. Noteworthy was the apparent ability of a low concentration of sufentanyl (12.5 nM) to displace a large percentage (70%) of naloxone binding in areas rich in either kappa (amygdala) or delta (striatum) binding subtypes. Our data suggest three possible conclusions: 1) while sufentanyl exhibits selective binding to mu sites in rat brain homogenates in $Na^+$-free buffers, the selectivity is largely reduced in $Na^+$-containing buffers with intact tissue, 2) under our binding conditions, naloxone itself binds exclusively to the mu binding site, or 3) only one high affinity opioid binding activity exists in intact rat brain when exposed to physiological $Na^+$ concentrations.

220.7 ONTOGENESIS OF μ-OPIOID RECEPTOR IN CHICK BRAIN. T. Geladopoulos and A. Vernadakis (SPON: N. Sakellaridis). Depts. of Pharmacology and Psychiatry Univ. Colo. Sch. Med., Denver, CO 80262.

Previous studies from this laboratory have shown stereospecific $^3$H-etorphine binding during early embryogenesis in the chick (Dev. Brain Res. 4:23-29, 1982). In this study we report the binding patterns of $^3$H-dihydromorphine ($^3$H-DHM, a selective μ agonist). $^3$H-DHM binding was studied at days 5,6 and 20 of embryonic age. At days 5 and 6 whole brain was used, and at day 20 cerebral hemispheres. Binding assays were performed by filtration. Non-specific binding was determined in the presence of $5 \times 10^{-7}$M levorphanol. Data were analysed using a Morrow Microdecision computer. Scatchard analysis of saturation curves (0.05-10 nM) revealed a single binding site for $^3$H-DHM at day 20. The $K_d$ and the $B_{max}$ were 1 nM and 50 fmol/mg protein, respectively. However, at day 5 and 6 a biphasic binding pattern of $^3$H-DHM was found. The $K_d$ for the high affinity site was 0.25 nM for both day 5 and day 6. The $B_{max}$ was 6 fmol/mg protein at day 5 and 14 fmol at day 6, reflecting an increase in binding sites that appear at day 6 with the affinity remaining constant. The low affinity binding undergoes changes in both affinity and number of binding sites. At day 5 the $K_d$ was 1.3 nM, the $B_{max}$ 5 fmol, and at day 6, 2.5 nM and 30 fmol, respectively. These data indicate the $^3$H-DHM binds to more than one receptor site during different developmental stages. Displacement binding studies were performed using various cold ligands. Levorphanol had a more pronounced inhibitory effect at day 20 than at days 5 and 6, whereas D-Ala$^2$-D-Leu$^5$-enkephalin (DADL, a δ specific agonist) showed more pronounced inhibition at days 5 and 6. These data indicate that by 20 days, the μ receptor is mature, and that during early embryogenesis the $^3$H-DHM binding site responds to both μ and δ ligands in an atypical manner. Furthermore, we propose that the low affinity binding may represent δ receptor. Additional saturation binding studies were performed in the presence of $5 \times 10^{-7}$M levorphanol and 1 μM DADL in order to possibly eliminate the low affinity binding. At day 20, the $^3$H-DHM binding was nearly unaffected by the presence of DADL, supporting the idea for an already mature μ opioid receptor. The high affinity binding at day 5 and day 6 was also not affected by the presence of DADL. However, the $K_d$ of the low affinity binding was dramatically increased. These results indicate that during early development the opioid receptor might not be differentiated to distinguish ligands assigned to μ and δ receptors in the adult. The functional role of the various types of opioid receptors during early embryogenesis remains to be elucidated. However, it is suggested that low affinity opioid binding sites may be involved in cell differentiation. (Supported by Research grant HD 18894 from NICHD)

220.8 SPECIFIC BINDING OF [$^3$H]-PHENCYCLIDINE TO RAT BRAIN MEMBRANES. Peter J. Egofske* and Ronald L. Kochman. Dept. of Biological Research, G.D. Searle & Co., Skokie, Ill. 60077.

The binding of [$^3$H]-phencyclidine (PCP) to rat brain synaptic membrane preparations (P2) has been studied. Kinetic binding data for [$^3$H]PCP, obtained by both rapid filtration and centrifugation methods, indicate multiple binding sites for [$^3$H]PCP. The binding of [$^3$H]PCP to rat brain membranes was found to be dependent upon homogenate protein concentration, pH, temperature, buffer, and ionic strength. Using a rapid filtration method, the specific binding of 10nM [$^3$H]PCP was found to be a linear function of homogenate protein concentration between 0.1 and 0.7mg/ml and maximal binding was obtained between pH 7.8 and 8.8 for 5mM TRIS-HCl buffer (37° C). Saturation was complete within 20 minutes when the incubation was carried out at 25° or 37° C; however, at 0° C, saturation was not complete until after 45 minutes. At equilibrium, specific binding at 0° C decreased by 50% when compared to the 25° or 37° C incubations. Specific binding of [$^3$H]-PCP decreased by a factor of two when the concentration of 5mM TRIS-HCl was increased to 50mM. After [$^3$H]PCP was incubated for 130 minutes at 37° C, greater than 95% of the bound radioactivity was determined to be intact drug. Heat-denaturation of the membranes destroyed the specific binding of [$^3$H]PCP. Competition studies between mu and sigma opiate ligands, PCP analogs and unlabeled PCP, against [$^3$H]PCP were carried out, using the rapid filtration method.

220.9 THE EFFECT OF MET-ENK VERSUS MORPHINE ON VARIOUS ASPECTS OF PHYSICAL DEPENDENCE. K.A. Steece, R.F. Ritzmann, J.M. Lee, J.Z. Fields, and F.A. DeLeon-Jones. West Side and Hines VA Med Ctrs, Chgo, IL, 60612.

Morphine and enkephalins act via different receptors, mu (u) and delta (S), respectively. Chronic administration of either morphine or methionine enkephalin (met-enk) results in development of physical dependence (p.d.). Data suggests there are some differences in p.d. produced by morphine and met-enk with regards to withdrawl symptoms as well as certain neurochemical systems. In particular, chronic met-enk treatment appears to up-regulate D2-DA receptors in the hypothalamus which positively correlates with an increased sensitivity to apomorphine(APO)-induced hypothermia. This is similar to that observed in chronic morphine treated animals. In the striatum, in contrast to morphine treatment where there is an up regulation and increased sensitivity to DA agonists, neither striatal D2-DA receptors nor APO-induced stereotypy appears different in chronic met-enk treated animals. Acute administration of 3H-met-enk (icv), with and without enzyme inhibitors, suggests that there are no significant differences in met-enk distribution in these two areas which may account for these differences. It has been demonstrated that opioid peptides induce S receptor down-regulation in vitro. On the other hand, it is generally agreed that morphine does not have a similar affect on the u or S receptors. Thus it is of interest to determine whether S receptor down-regulaion in vivo occurs with chronic administration of met-enk and if so, if the time course of change follows the development of the symptoms of p.d.. The differences in morphine and met-enk regulation of u and S receptors, respectively, may account for some of the differences observed in the symptomologies of p.d. on these compounds. In addition, since both of these areas are abundunt in S receptors, there may be a difference in regulation of S receptors in the striatum and hypothalamus. Preliminary studies suggest that there is no change in striatal S receptors following chronic treatment with met-enk. However, this does not rule out the possibility that there may be a change in ligand recogition site regulation beyond the receptor per se, ie: N-protein or cation effect.

220.10 NALBUPHINE: AN AUTORADIOGRAPHIC OPIOID RECEPTOR BINDING PROFILE OF AN AGONIST/ANTAGONIST ANALGESIC. W.K. Schmidt*, E.B. De Souza and M.J. Kuhar (SPON: B.E. Lerer). Lab of Neuroscience, Addiction Research Center, NIDA, Baltimore, MD 21224, Dept. of Neuroscience, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205, and Pharmaceuticals Research and Development Div., Du Pont Pharmaceuticals, E.I. du Pont de Nemours, Willmington, DE 19898.

Nalbuphine is a potent analgesic with a low side effect and dependence profile in animals and man. Results from previous studies suggest that nalbuphine is primarily a kappa-agonist/mu-antagonist analgesic (Schmidt et al., Drug and Alcohol Dependence 14:339, 1985). The present study was designed to examine the characteristics of nalbuphine binding to mu, delta and kappa opioid receptors and to localize the sites of action of nalbuphine in the CNS using in vitro labeling light microscopic autoradiography.

Mu, delta, and kappa receptors were selectively labeled with 1.8 nM $^3$H-dihydromorphine, 4 nM $^3$H-D-ala$^2$-D-leu$^5$-enkephalin in the presence of 30 nM morphine, and 1.6 nM $^3$H-(-)ethylketocyclazocine in the presence of 30 nM morphine and 100 nM D-ala$^2$-D-leu$^5$-enkephalin, respectively. In displacement studies in rat brain homogenates, nalbuphine had the highest affinity ($K_i$; mean $\pm$ SEM) for mu receptors (0.46 $\pm$ 0.06 nM) with progressively lower affinities for kappa (29.0 $\pm$ 6.0 nM) and delta (59.5 $\pm$ 5.2 nM) receptors. In autoradiographic studies in slide-mounted sections of guinea pig brain and monkey spinal cord, nalbuphine (300 nM) completely displaced the binding at mu and kappa receptors without significantly altering the binding at delta receptors.

Next, we studied the autoradiographic distribution of $^3$H-nalbuphine binding sites in guinea pig and monkey CNS. Preliminary experiments were carried out in slide-mounted sections of guinea pig forebrain in order to determine the optimal binding parameters and to characterize the binding of $^3$H-nalbuphine in brain. The binding of $^3$H-nalbuphine was saturable and showed a curvilinear profile indicating the presence of two binding sites with apparent $K_d$ values of 0.52 and 11.4 nM. Morphine and U-50,488H which have high affinities for mu and kappa receptors, respectively, inhibited $^3$H-nalbuphine binding with $IC_{50}$ values of 0.9 and 10 nM, respectively. In saturation studies, morphine (50 nM) and U-50,488H (100nM) selectively eliminated the high and low affinity components of $^3$H-nalbuphine binding, respectively. The autoradiographic distribution of $^3$H-nalbuphine binding sites in the CNS corresponds well to the distribution of mu and kappa receptors. In addition, CNS areas (Laminae I and II of the spinal cord, substantia gelatinosa of the trigeminal nerve, periaqueductal grey, and thalamic nuclei) that mediate analgesia contain high concentrations of $^3$H-nalbuphine binding sites. In summary, these data demonstrate that nalbuphine acts on mu and kappa opioid receptors and provide anatomical loci in the CNS where nalbuphine may produce its actions. Supported by DA00266, NS15080 and MH00053.

220.11 REPEATED ELECTROCONVULSIVE SHOCK DOWN-REGULATES THE OPIOID RECEPTORS IN RAT BRAIN. Y. Nakata[1], K.J. Chang[2], C.L. Mitchell, and J.S. Hong[1]. [1]Lab. Behav. Neuro. Toxicol., NIEHS/NIH, Research Triangle Park, NC 27709, [2]The Wellcome Research Laboratories, Research Triangle Park, NC 27709.

It has been suggested that endogenous opioid peptides may mediate electroconvulsive shock (ECS)-elicited behavioral alterations, such as analgesia, retrograde amnesia, changes in seizure threshold or postictal depression. This notion is supported by our previous report that repeated ECS increases the [Met$^5$]-enkephalin-like immunoreactivity (ME-LI) in certain limbic areas of the rat brain, such as septum, amygdala, nucleus accumbens and hypothalamus whereas the level of hypothalamic β-endorphin remains unaltered. Our recent study using in vitro cell free translation or blot hybridization using a rat preproenkephalin A cDNA clone to estimate the level of mRNA coding for preproenkephalin A suggests that the increase in hypothalamic ME-LI after repeated ECS is due to an increase in biosynthesis. Recently, we observed that levels of dynorphin A (1-8)-like immunoreactivity (DN-LI) in several brain regions are also greatly affected after 10 daily ECSs: Namely, an increase in hypothalamus, caudate nucleus, septum and a decrease in hippocampus. These observations suggest that the metabolism of these two opioid peptide-containing neurons can change in response to repeated seizures. The purpose of this study was to determine the possible changes of opioid receptors which may occur as a consequence of the activation of enkephalin and dynorphin systems after repeated ECS. An in vitro receptor binding technique was used to monitor the changes in receptors.

Ten consecutive daily ECS delivered to the rats through ear-clip electrode with the following parameters: 85 mA, 50 Hz, 1 msec pulse interval for a total duration of 1 sec, which produce maximal tonic and clonic convulsions, caused reductions of mu and delta opioid receptor binding in hypothalamus, hippocampus, and caudate nucleus, but not in frontal cortex and brain stem. These changes of opioid receptor binding were not observed in rats receiving a single ECS. Scatchard analysis revealed that ECS-induced reduction of mu and delta receptor binding was due to a decrease in the binding sites but not to a change in the binding affinity. Time course studies showed that 7 days after the end of 10 consecutive daily ECSs, both mu and delta receptor binding remained lower than those of sham-controls. However, the effects of ECS on the opioid receptor binding disappeared in 2-3 weeks. These observations are consistent with the hypothesis that ECS treatments increase the release of opioid peptides in certain brain regions which in turn down-regulate the opioid receptors.

220.12 DISTRIBUTION OF OPIATE RECEPTOR SUBTYPES AND ENKEPHALIN AND DYNORPHIN IN THE HIPPOCAMPUS OF SQUIRREL, GUINEA PIG, RAT, AND HAMSTER. (Sponsor: J. Heym) S. McLean*[1], R. Rothman[3], K. Rice*[2], A. Jacobson*[2] and M. Herkenham[1]. [1]Lab. Neurophysiol, NIMH, [2]Lab. Chemistry, NIADDK, Bethesda, MD, 20205 and [3]Lab. Preclinical Pharmacol., St. Elizabeths Hospital, NIMH, Washington, DC 20032.

The distribution of mu, delta, and kappa opiate receptor subtypes was compared to the immunocytochemical distribution of dynorphin B and enkephalin in the hippocampus of four rodent species: squirrel, guinea pig, rat and hamster. For immunocytochemical determinations, 30 μm-thick sections from paraformaldehyde-fixed brains were incubated with dynorphin B or enkephalin antisera and processed by the avidin-biotin complex method. The three major opiate receptor subtypes were localized by in vitro binding in unfixed slide-mounted tissue sections utilizing binding conditions for selective and maximal occupation of mu, delta and kappa sites by [$^3$H]D-ala-$^2$MePhe$^4$-Gly-ol$^5$-enkephalin, [$^3$H]D-ala$^2$-D-leu$^5$-enkephalin and [$^3$H]bremazocine, respectively. Selectivity for the mu site is derived from the specificity of the ligand itself. For delta sites 30 nM oxymorphone was included in the incubation medium to block high affinity binding to the mu site. Sections enriched in kappa sites were preincubated with etonitazene and fentanyl derivatives, BIT and FIT, agents that irreversibly alkylate the mu and delta sites, respectively, then incubated with [$^3$H]bremazocine at 4°C in the presence of 0.4 M NaCl. Following binding the tissue was washed, blown dry and apposed to LKB Ultrofilm for two months.

Dense labeling of fibers immmunoreactive for enkephalin and dynorphin-like material is present in the mossy fiber system of all species. Occasional fibers reactive for enkephalin or dynorphin are seen in most layers of the hippocampus.

In all species, mu and kappa receptors are similarly but not identically distributed in the hippocampus, predominantly in the molecular layer of the dentate gyrus and pyramidal cell layer of CA3 and CA4. Kappa binding in the mossy fiber sytem is dense in the squirrel and guinea pig but sparse in the rat and hamster. In contrast to the mu and kappa patterns, delta receptors are lowest in the pyramidal and granule cell layers and in the mossy fiber system of all four species. Delta receptors overlap with the mu and kappa receptors in the dentate molecular layer, but are more extensively distributed in stratum radiatum and stratum oriens.

Whereas the patterns of opiate receptor subtypes show interspecies variability, the opioid peptides do not. Furthermore, the opiate receptor subtypes are more extensively distributed throughout the hippocampus than are the peptides, and the laminar patterns of receptor binding bear no consistent relationship with the distribution of endogenous opiate-like material.

220.13 [$^3$H]Naloxone Autoradiography: Distribution of Lambda Opiate Binding Sites in Rat Brain. David C. Perry[1] and Wolfgang Sadee[2], Department of Pharmacology[1], George Washington University Medical Center, Washington, D.C., 20037, and Department of Pharmaceutical Chemistry[2], University of California, San Francisco, CA, 94043.

Recent in vivo and in vitro studies describe an opiate binding site in rat brain (the lambda site) which is distinct from the previously described mu, delta, kappa, or sigma sites (Grevel and Sadee, Science 221: 1198, 1983). We have employed in vitro receptor autoradiography techniques with [$^3$H]naloxone to determine the distribution of this site in rat brain. Frozen 10 µm sections were incubated for 20 minutes at 4 C with 4 nM [$^3$H]naloxone, then rinsed for 3 minutes with ice-cold buffer, dried and exposed to tritium-sensitive film to visualize bound radioactivity. Non-specific binding was defined as that remaining in the presence of 1 µM naloxone; lambda sites as those remaining in the presence of 300 nM diprenorphine (Grevel and Sadee, 221: 1198, 1983). Lambda sites were widely distributed throughout the brain, showing a unique pattern of distribution. Total binding (no drugs added to incubation) resembled previously published reports for the distribution of mu opiate receptors. Lambda site distribution was significantly different; many areas enriched in mu sites showed little or no lambda binding. The major regions showing significant lambda binding were the cortex (esp. laminae I and II); the amygdala; the cerebellar gray matter; several thalamic nuclei; and the hippocampus. Lambda sites in the first four regions are relatively evenly distributed: [$^3$H]naloxone labeled both mu and lambda sites, in varying proportions, but with generally similar distribution within the region. The finding in the hippocampus was more striking, however. An intensely labeled band was seen which appeared to consist almost entirely of lambda sites; this band was congruent with the mossy fiber tract (i.e., pyramidal cell layer of the dentate gyrus and fields CA3 and CA4). This localization of lambda sites may shed some light on their possible function; opiates exhibit unique excitatory effects on the pyramidal cells of the hippocampus, and the mossy fiber tract demonstrates intense dynorphin immunoreactivity (Chavkin, et al., J. Neurosci 5: 808, 1985).

## PROCESS OUTGROWTH: MOLECULAR MECHANISMS

221.1 CHANGES IN THE PHOSPHORYLATION AND DISTRIBUTION OF VINCULIN DURING NERVE GROWTH FACTOR INDUCED NEURITE OUTGROWTH. Simon Halegoua*, (SPON:D.Deutch) Dept. of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, N.Y. 11794.

Nerve growth factor (NGF) treatment of PC12 cells was used to study the mechanism of neurite initiation and elongation. The distribution of focal adhesion sites and of the cytoskeletal protein, vinculin, was determined in large fused, multinucleated PC12 cells. In the absence of NGF, focal adhesion sites were restricted to the cell periphery in an even distribution as seen by interference reflection microscopy. By indirect immunofluorescence microscopy using affinity purified anti-vinculin antibodies, vinculin assembly (foci) was also found to be restricted to the cell periphery apparently at focal adhesion sites. Within four hours after NGF treatment of the cells, the distribution of both vinculin and focal adhesion sites began to change. Focal adhesion sites became further restricted to discrete protruding portions of the cell periphery. Immunofluorescence localization of vinculin displayed larger, brighter vinculin foci at the tips of the cell margin extensions, concommitant with the loss of foci at locations between the protrusions. As the formation of neuritic growth cones proceeded from discrete locations at the cell margin, both focal adhesion sites and vinculin foci remained with the tips of growth cone extensions. Both focal adhesion sites and vinculin foci were rarely seen in the perikarya of cells with elongating neurites, and these were always confined to extending portions of the cell body margin. Occassionally, vinculin foci could be seen at the proximal protruding portion of the neurite and at discrete locations along the neurite, usually at protruding tips along the legnth. By immunoprecipitation of vinculin from $^{32}$P labeled cells, vinculin was also found to become more heavily phosphorylated within one hour of NGF treatment. Based upon these and previously reported data on neurite extension, a model is presented for the mechanism by which NGF directs the formation and elongation of neuritic processes.

221.2 NGF-SENSITIVE PHOSPHOPROTEINS FROM PC12 CELLS ARE DIFFERENTIALLY LOCALIZED BETWEEN CELL BODIES AND NEURITES. J.M. Aletta and L.A. Greene*. Dept. Pharmacology, NYU Med. Cen., New York, NY 10016.

We are interested in identifying and characterizing the molecules involved in the growth, motility, and structural maintenance of neurites. In order to achieve this goal, we have developed an isolated neurite preparation from NGF-treated PC12 cells. Cells are initially plated within small cloning cylinders placed on collagen-coated culture dishes to produce small discrete clusters which form an extensive radiating neuritic network during exposure to NGF for 3-5 weeks, at which time the cell bodies can be surgically excised (see Estridge and Bunge JCB 79: 138, 1978).

By metabolically labeling such cultures with [$^{32}$P]-ortho-phosphate, we have found that the relative levels of several phosphoproteins are remarkably enriched in the neurites compared to unfractionated cells cultured with NGF as above or in dispersed monolayers, or compared to the excised cell bodies. Specifically, five such phosphoproteins ($M_r$ 55, 64, 72, 80, and ~ 300 kd) are consistently detected by SDS PAGE and 2-D IEF SDS PAGE. This result is obtained whether the 1 hr labeling period is carried out before or after removal of the cell bodies. In separate experiments with unfractionated PC12 cell cultures we have observed that long term NGF treatment is a prerequisite for the detection of each of these phosphorylated forms in substantial quantity. Various criteria indicate that the 55 kd phosphoprotein is a tubulin, while the 64, 72, 80, and 300 kd species are microtubule-associated proteins. The 55 kd protein comigrates with the tubulin complex on 2-D gels and phosphorylation of at least the 55 and 64 kd proteins is markedly reduced in cultures treated with the microtubule depolymerizing agents nocodazole or podophyllotoxin, 50 µM, for 2-15 hrs. Drug treatments, however, do not produce visible deterioration of neurites or their attachment to the substrate. This indicates that these two phosphorylated forms are not likely to be required to stabilize the overall structure of the neurite.

Further analysis of the 2-D gels has revealed several other phosphoproteins preferentially localized to neurites. Of particular interest are two protein spots present only in samples from NGF-treated PC12 cells. Withdrawal of NGF from neurite bearing cultures for 6 hrs prior to harvesting results in their dephosphorylation. Readdition of NGF to sister cultures for the last 1/2 hr of the 6 hr period, however, is sufficient to allow re-phosphorylation of these two substrates. These are candidates for growth related functions since the withdrawal and readdition of NGF are known to produce rapid effects on the motility and structure of PC12 neurites and growth cones. Supported by grants from March of Dimes, NIH (NS16036) and a postdoctoral fellowship to JMA from the Muscular Dystrophy Association.

221.3 HETEROGENEOUS DISTRIBUTION OF LECTIN BINDING SITES ON THE CELL SURFACES OF DEVELOPING CENTRAL NERVOUS SYSTEM NEURONS. K.L. Lankford* and W.L. Klein. (Spon: B. Menco) Dept. Neurobiology and Physiology, Northwestern University, Evanston, IL 60201.

In order to investigate the overall organization of the plasma membrane of developing neurons, wholemounts of cultured embryonic chick retina neurons were labeled with lectin-conjugated 10 nm gold colloidal particles and examined with transmission electron microscopy. This technique provided a complete overview of the distribution of lectin binding sites with high resolution localization of specific labeling.

A heterogeneous distribution of wheat germ agglutinin-gold colloidal particle (WGA-GCP) labeling was observed in both pre-fixation and postfixation labeled specimens. The heterogeneous pattern of labeling was apparent in both serum supplemented and defined medium cultures. The density of labeling was highest on filopodia (285 WGA-GCPs/$um^2$) and lamellipodia (264 WGA-GCPs/$um^2$), but significantly lower on the growth cones themselves and the cell bodies (125 WGA-GCPs/$um^2$). In addition, patches of high density labeling could be detected within each region. In a representative growth cone region, 40% of the label was contained within 14% of the total cell surface area. Labeling tended to be particularly dense on the tips of filopodia, supporting the theory that glycoproteins mediate filopodial adhesion. Preliminary results suggest a similar pattern of distribution of Concanavalin A binding sites. The pattern of lectin labeling suggests that morphologically and functionally discrete regions of the cell surface are biochemically different.

The procedures utilized here for localization of lectin binding sites can also be applied to antibody labeling techniques. Labeling of wholemounts using gold-conjugated secondary antibodies should provide useful insight into the distribution of known neuronal antigens.

(Supported by NIH grant NS21088 to W.L. Klein.)

221.4 **CHARACTERIZATION OF A NEURONAL PROTEIN THAT BINDS PLASMINOGEN ACTIVATOR.** Randall N. Pittman. Department of Pharmacology G3, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

A urokinase-like plasminogen activator is spontaneously released by distal processes and growth cones of cultured rat sympathetic neurons (Pittman, Dev. Biol., in press). After being released, the neuronal plasminogen activator binds to a protein on the surface of sympathetic neurons. The complex between plasminogen activator and the surface protein is irreversible and has a MW of 68 kDa.

PC 12 pheochromocytoma cells treated with nerve growth factor (NGF) also have a protein on their surface that forms a 68 kDa complex with plasminogen activator. A MW of 50 kDa was determined for the PC 12 protein by solubilizing NGF-treated PC 12 cells followed by SDS gel electrophoresis, blotting onto nitrocellulose and probing the nitrocellulose with $^{125}$I-urokinase. This same protein not only binds plasminogen activator but can also bind (via a different site) to a component in the extracellular matrix (ECM). This was determined by solubilizing membranes from NGF-treated PC 12 cells with Tween-20 followed by exposing the solubilized proteins to extracellular matrix from bovine corneal endothelial cells. The matrix was washed extensively with isotonic Tris followed by washes of increasing salt concentrations. Whereas all proteins detected in Coomassie- or silver-stained gels were eluted in 0.1-0.2 M NaCl, the 50 kDa protein (detected by its binding to $^{125}$I-urokinase) eluted from the matrix between 0.4 and 0.6 M NaCl, indicating a strong binding to a component in the ECM.

Experiments are currently in progress to determine if the 50 kDa surface protein which binds components of the ECM as well as plasminogen activator is involved in process outgrowth, to determine if the binding of plasminogen activator to this protein alters its affinity for the ECM, to identify the component(s) of the ECM to which it binds, and to produce immunological probes to the 50 kDa protein.

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221.5 DEVELOPMENTAL REGULATION OF A GROWTH CONE MEMBRANE PROTEIN, GAP-43, IN RAT CNS. R. D. Jacobson and J. H. P. Skene, Dept. of Neurobiology, Stanford University, Stanford, CA 94305.

Axonal growth in several types of neurons has been shown to be associated with increased synthesis of a few neuronal proteins which are transported rapidly into axons (Skene and Willard, J. Cell Biol. 89: 86, '81; Benowitz et al., J. Neurosci. 3: 300, '81; Skene and Kalil, Neurosci. Abstr. 10: 1030, '84). To determine whether synthesis of similar growth-associated proteins is a general characteristic of developing CNS neurons, we have studied one growth-associated protein, GAP-43, in developing and adult rat brain. Rat CNS proteins were radiolabelled in vivo by intracerebral injection of $^{35}$S-methionine, cerebral cortex and cerebellum were removed and homogenized, and 100,000g particulate fractions were subjected to 2D gel electrophoresis. Proteins were visualized by Coomassie Blue staining, to measure steady-state abundance, and fluorography, to measure rate of synthesis.

A heavily labeled protein from both cortex and cerebellum of neonatal rats co-migrates on two-dimensional gels with GAP-43 from regenerating toad optic nerves. This rat "GAP-43" has a pI of approximately 4.6 and an apparent molecular weight of about 47K on 12% polyacrylamide gels. The apparent molecular weight of both the toad and rat proteins decreases with increasing acrylamide concentrations, suggesting atypical SDS binding, or unusual shape, or both. Both synthesis and abundance of GAP-43 remain high during the first two postnatal weeks, then decline over the next six weeks to trace, but still detectable, levels in adults. In vitro translations of cortex poly-$A^+$ RNA from neonatal animals yields a protein with a similar pI, and apparent molecular weights changing with gel concentrations, as GAP-43. Labeling of this protein is much higher after translation of neonatal cortex RNA than after translation of a similar amount of RNA from adult cortex, indicating that much of the developmental regulation of GAP-43 synthesis occurs through specific changes in GAP-43 mRNA.

To confirm that GAP-43 in the rat CNS is neuronal and axonally transported, we prepared purified growth cones from neonatal and fetal rats (Pfenninger, et al., Cell 35: 375, '83). GAP-43 was highly enriched in a growth cone membrane fraction, compared with whole brain homogenates. Similarly, the small amount of GAP-43 in adult brains was enriched in synaptosomal membrane preparations. Growth cone membranes contain an order of magnitude more GAP-43 than adult synaptosomal membranes, supporting the hypothesis that the protein plays a role in axon elongation. However, the presence of significant amounts of GAP-43 in adult synaptic terminals suggests an additional function.

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221.6 EXPRESSION OF pp60-src PROTO-ONCOGENE IN DEVELOPING RAT AND REGENERATING FISH CENTRAL NERVOUS SYSTEM. J. G. Steedman* and G. E. Landreth (SPON: D. Cotanche). Departments of Anatomy and Neurology, Medical University of South Carolina, Charleston, SC 29425.

The proto-oncogene, pp60-c-src, whose retroviral homolog is the transforming factor of Rous sarcoma virus, is expressed at high levels in the vertebrate nervous system. Using a monoclonal antibody to the viral oncogene, we have investigated the expression of pp60-src in the embryonic rat brain and in the regenerating optic nerve of goldfish by assaying for tyrosine kinase activity of immunoprecipitated protein, by Western blot analysis of $I^{125}$-labelled immune complex, and by immunohistochemical localization under the light microscope.

The activity of pp60-src in the developing rat brain rose from embryonic day 12 (E12) to a maximum at E16-17, thereafter dropped steadily through early postnatal life and was lowest in adult brain. Activity was found to be highest in forebrain relative to other areas. Immunohistochemical localization of pp60-src was in accord with these findings. At E16, pp60-src was preferentially localized in a band in presumptive cerebral cortex in the region of the newly forming cortical plate. Reaction product was located in processes of these actively migrating cells, and was not observed either in nuclei or subventricular or marginal zones. Earlier and later stages of development failed to show reaction product above background, suggesting that at present our methods may not be sensitive enough to localize pp60-src when expressed at lower levels.

In retinae of goldfish whose optic nerves were regenerating following optic nerve crush, identical methods showed elevated levels of pp60-src expression, rising to a peak of activity at 7-8 days.

In other cell types, pp60-src causes the appearance of cell-surface ruffles suggesting the induction of cytoskeletal reorganization by pp60-src. The extension of filopodia and neurites by newly differentiated or regenerating neurons is a feature of normal cell activity that involves such cytoskeletal reorganization. Our hypothesis is that the comparatively high levels of this proto-oncogene activity seen in the developing and regenerating nervous system reflect cell-surface mediated events associated with the elaboration of neuritic processes.

221.7 EFFECTS OF 12-O-TETRADECANOYLPHORBOL-13-ACETATE(TPA) ON AXONAL ELONGATION IN SENSORY GANGLIA - AN ELECTRON MICROSCOPIC STUDY. L. Hsu and E. Zycband*, Dept. of Anatomy, UMDNJ-School of Osteopathic Medicine, Piscataway, NJ 08854.

Previously we reported on the neurite-promoting effects of the phorbol ester tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) in chick sensory ganglia explants (Hsu et al, Cancer Res., 44:4607-4614, 1984). The addition of TPA to serum-less growth medium which did not contain Nerve Growth Factor enhanced the differentiation of ganglionic neurons promoting the development of the rough endoplasmic reticulum and Nissl substance. The Golgi complex within these neurons was greatly enlarged and a significant increase in cytoplasmic microtubules and neurofilaments was evident. At low (10 ng/ml) concentrations of TPA, the tumor promoter elicited the rapid outgrowth of long, radial neurites. Along the lengths of these axons numerous varicosities were encountered which were filled with vesicular and cytoskeletal components. These presumably were depots of membranous reserves occurring at sites of extension. Distally, growth cones and their motile filopodial regions were also prevalent. In contrast, explants maintained in control medium without TPA developed much fewer of these structures. With high (100 ng/ml) concentrations of TPA neurite outgrowth was very dense with the formation of thick but short fascicles. From the explant core, axons extended and merged to form short, compact bundles. The axons within such fascicles were distinctively wider than those found in control explants or explants treated with low concentrations of TPA. Microtubular bundles were the dominant components within these axons which were congested with a variety of vesicular profiles including many autophagic vacuoles. Growth cones or filopodial regions were conspicuously absent. In explants treated with high concentrations of TPA degenerative changes involving the formation of deep clefts in some of the neuronal soma and the appearance of dense matrix in certain axons were observed.

Our results suggest that alterations in axonal morphology by TPA was dose-dependent. At low concentrations, TPA promoted the development of membranous and motile structures associated with the elongation process. In contrast, as a result of the dense outgrowth of neurites produced by high concentrations of TPA, formation of thick fascicles led to the retraction of axons and the cessation of elongation.

This work was supported by the New Jersey State Commission on Cancer Research.

221.8 **A NOVEL ROLE FOR SEROTONIN AND OTHER NEUROTRANSMITTERS: THE CONTROL OF NEURITE OUTGROWTH.** S.B.Kater, P.G.Haydon, A.D.Murphy* and D.P.McCobb. Department of Biology, University of Iowa, Iowa City, IA 52242. *Department of Medical Physiology, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

The characteristic morphology of individual neurons is a result of the combined actions of a variety of extrinsic signals upon the intrinsic properties of the individuals. Such extrinsic signals act to promote, guide and finally to halt neurite extension. Recently we have demonstrated in cell culture experiments that serotonin has the potential to act as an extrinsic regulatory agent of outgrowth cessation (Haydon et al, Science 226: 561, 1984; J. Neurosci. Res. 13: 135, 1985). This neurotransmitter selectively inhibits active neurite extension and growth cone motility of specific identified neurons of Helisoma. By acting locally at the growth cone, serotonin inhibits the outgrowth of neuron 19 while having no effect on neuron 5. However, since the environment that growth cones encounter in cell culture is clearly different from that in nerve trunks, for example, it is important to determine whether serotonin is capable of such an action within the nervous system in the presence of processes such as transmitter uptake and degradation. Accordingly, we placed the nervous system of Helisoma into organ culture where, following axotomy, neurons extend neurites at a reliably constant rate. Under such conditions the addition of serotonin to the bathing medium for the duration of culture (2 days) significantly and selectively reduced the extent of outgrowth attained by specific neurons as determined by the intracellular injection of Lucifer Yellow. In situ the extent of neuron 19's outgrowth was significantly reduced by the presence of serotonin, whereas the outgrowth of neuron 5 was unaffected. In addition, the outgrowth of the identified neuron C1 was assayed. Just as with neuron 19, the outgrowth of C1 was significantly inhibited by the presence of serotonin. Thus in situ serotonin selectively inhibits the outgrowth of specific identified neurons.

In preliminary experiments performed to assess the potential involvement of cyclic AMP in mediating serotonin's inhibitory effects on outgrowth, the action of the potent activator of adenylate cyclase, forskolin, was tested in cell and organ culture experiments. Forskolin mimicked the effect of serotonin on neuron 19, causing an inhibition of neurite outgrowth. Furthermore, forskolin3 caused an inhibition of neurite extension from neuron 5 despite the fact that this neuron was unresponsive to serotonin. This indicates that the extension of neurites from neuron 5 is capable of being inhibited, and suggests that neurotransmitters other than serotonin may have this action on neuron 5. Taken together our data point towards a role for neurotransmitters in regulating the formation of neural architecture in addition to acting in their classical role of synaptic transmission.

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221.9 **DOPAMINE: AN ADDITIONAL REGULATOR OF NEURITE OUTGROWTH IN *HELISOMA*.** D.P. McCobb, P.G. Haydon, and S.B. Kater. Dept. of Biology, University of Iowa, Iowa City, IA 52242

Serotonin has striking inhibitory effects on regenerative neurite outgrowth from specific identified neurons of *Helisoma* buccal ganglia (Haydon et al, Science 226: 561, 1984, Haydon et al, J Neurosci Res 13: 135, 1985). Dopamine is endogenous to the buccal ganglia and, like serotonin, has been shown previously to elicit motor program activity. This study tests whether dopamine can also regulate neurite outgrowth of buccal ganglion neurons.

Identified buccal ganglion neurons were placed in isolated cell culture. The advance of individual growth cones over the substratum was monitored photographically for two hours before and after addition of neurotransmitters to the culture medium in bolus dose.

**Percentage of Growth Cones Inhibited by Serotonin and Dopamine**

| | 5-HT | DA |
|---|---|---|
| neuron 5 | 0 | 0 |
| neuron 19 | 93.3 | 76.5 |
| neuron P5 | 100 | 0 |

Growth cones of neuron 19 initially respond to dopamine and serotonin similarly. The above data for DA are at 50 µM; at 1 µM DA inhibited 60% of neuron 19's growth cones. The inhibition is accompanied by morphological changes at the growth cone, often including a sharp reduction in filopodial number. Individual growth cones on a single neuron 19 may exhibit different responses to DA; most may stop while some are apparently unaffected. Thus sensitivity to the agent differs locally on a neuron. Unlike neuron 19 growth cones in the presence of serotonin, which are permanently inhibited, neuron 19's growth cones in dopamine may recover from the inhibitory effects. Even in the continual presence of 50 µM DA some neurons will resume outgrowth within 2 hours. This transient effect, as opposed to a long lasting inhibition, is very similar to the response of growth cones of neuron P5 to serotonin. As is true with synaptic transmission, the nature of a transmitter's action is governed by the target cell rather than the transmitter itself. The developmental roles of serotonin and dopamine may involve permanently shaping dendritic arbors, or, in the case of some specific neurons, they may serve as more dynamic and transient regulators of growth cone traffic during growth cone migrations.

The sets of neurons afffected by DA and 5-HT are distinct. Serotonin inhibits outgrowth of neuron 19, has no effect on neuron 5, and transiently inhibits the outgrowth of pedal ganglion neuron P5. Dopamine effects neuron 19 as described, has no effect on neuron 5, and clearly has no effect on neuron P5. The two transmitters may therefore play distinct regulatory roles.

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221.10 ROLE OF SCHWANN CELLS AND THEIR EXTRACELLULAR MATRIX IN PROMOTING NEURITE GROWTH IN VITRO. M.D. Ard, R.P. Bunge and M.B. Bunge. Dept. Anat. & Neurobiol., Washington Univ. St. Louis, MO 63110.

The ability of Schwann cells to enhance the rate of growth of neurites has been tested in a tissue culture model of nerve regeneration. Implants were aggregates of fetal rat dorsal root ganglia (DRG) neurons (supporting cell free) which had been maintained in culture for 5-8 weeks. Recipients were model "injured nerves" created in vitro by growing a DRG explant on collagen for 4-6 weeks so that neurites of the extensive outgrowth were ensheathed and myelinated by Schwann cells. Then the explant was cut out, leaving the Schwann cells and their extracellular matrix (ECM) along with degenerating neurites in the culture dish. Three days later, the freshly axotomized implants were placed at the peripheral edge of the prepared outgrowth of Schwann cells and matrix. Regenerating neurites grew from the implant in all directions, some onto the prepared outgrowth and others onto bare collagen. Models of injured nerves were of four types: 1) organotypic nerves, in which DRG neurites were accompanied not only by ensheathing Schwann cells but also by fibroblasts (SC+ECM+FB); 2) Schwann cells ensheathing DRG neurites in the absence of fibroblasts but in the presence of culture medium adequate for formation of myelin, basal lamina and collagen fibrils (SC+ECM); 3) ECM remaining in cultures of type 2 after cells had been extracted with detergents (ECM only); and 4) Schwann cells ensheathing DRG neurites in incomplete culture medium which does not allow the formation of ECM or myelin by Schwann cells (SC only). At the end of 4 days of neurite regeneration, neurite lengths on the prepared outgrowth and on plain collagen were compared.

The results showed that Schwann cells with ECM, ECM alone, or Schwann cells without ECM promote neurite outgrowth. Using Wilcoxon's signed rank test at a significance level of 0.05, neurites growing among Schwann cells or Schwann cell ECM were shown to be longer than neurites growing on plain collagen. The median ratio of neurite length on prepared outgrowth to neurite length on bare collagen in each category was: 1) SC+ECM+FB, 1.8; 2) SC+ECM, 1.7; 3) ECM only, 1.6; and 4) SC only, 1.3. (In an additional experiment of type 2 (SC+ECM) in which transplantation was onto 1 day rather than 3 day prepared outgrowth, the median ratio of neurite length was 1.0.) Explants of embryonic rat cerebral cortex also showed significantly enhanced neurite growth on the prepared outgrowths. The neurite-promoting activity appears to be attached to cell surfaces and ECM rather than to diffuse throughout the culture medium. We conclude from this study that Schwann cell ECM alone is as effective in promoting outgrowth from peripheral and central neurons as is ECM accompanied by Schwann cells. (Supported by NIH grants NS 15070, NS 09923 and training grant 52252.)

221.11 RAT SCHWANNOMA NEURITE PROMOTING FACTOR IS A LAMININ-HEPARAN SULFATE PROTEOGLYCAN COMPLEX. G.E. Davis*, M. Manthorpe and S. Varon (Spon: J. Hatton). Dept. Biology, Sch. of Med., Univ. of Calif. San Diego, La Jolla, CA 92093.

Recent studies indicate that a substratum-binding neurite promoting factor produced by rat Schwannoma cells contains the extracellular matrix glycoprotein, laminin (Davis et al., J. Neurosci., in press) which itself has potent neurite promoting activity (Manthorpe et al., J. Cell Biol. 97: 1882-1890, 1983). However, it is evident that laminin is not the only extracellular matrix component present in the purified Schwannoma factor preparation. Several lines of evidence indicate that heparan sulfate proteoglycan is complexed with laminin in this factor. First, during its purification, a highly sulfated component, labelled with $^{35}SO_4$, copurifies with laminin antigens detected by ELISA. Second, nearly all of the sulfated material is degradable with nitrous acid suggesting that it is heparan sulfate. Third, the purified factor cross-reacts with antibodies directed against a basement membrane-derived heparan sulfate proteoglycan. The purified factor can also be distinguished from purified laminin due to its greater affinity for DE52 ion-exchange columns and greater buoyant density in cesium chloride gradients, both of which probably reflect the presence of proteoglycan.

Previous studies have indicated that purified laminin and heparan sulfate proteoglycan interact with one another primarily through ionically sensitive mechanisms (Fujiwara et al., Eur. J. Biochem. 143: 145-157, 1984). However, the Schwannoma factor complex may be held together by different (possibly hydrophobic or lectin-like) mechanisms since it is stable to very high salt (~2M CsCl) during cesium chloride gradients in 0.4M guanidine-HCl buffer. It is not clear whether this high affinity interaction is the result of a direct laminin-heparan sulfate proteoglycan interaction or through a "bridging" molecule which has high affinity for both components. By SDS-PAGE, two additional protein bands at approximately 150 and 35 kD are observed in the Schwannoma factor preparation. These currently unidentified protein bands may serve in such a "bridging" role. The 150 kD band might be related to the sulfated extracellular matrix protein, entactin (Carlin et al., J. Biol. Chem. 256: 5209-5214, 1981), which has a similar molecular weight and has also been found in association with laminin in different cell conditioned media. Since laminin appears to be the active neurite promoting protein in the Schwannoma factor complex, the additional components may be important for the proper binding and organization of biologically active laminin in vivo on cell surfaces and basement membranes.

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221.12 OUTGROWTH OF EMBRYONIC RETINAL CELL AXONS IN XENOPUS IS PROMOTED BY A FACTOR, PROBABLY NOT LAMININ, ON THE SURFACE OF A GLIAL CELL LINE. W.A. Harris. Department of Biology, University of California, San Diego, La Jolla, CA 92093.

By maintaining for many generations cells that grow out of explanted or dissociated embryonic Xenopus retinas, we have been able to grow several morphologically distinct cell lines. One such line (XR1) is composed of a cell type which closely resembles one that in primary cultures provides a very attractive membrane surface for neurite outgrowth. The similarity is both morphological and immunohistochemical for both are large flattened cells which stain heavily for Glial Fibrillary Acidic Protein but not Vimentin. In primary cultures, retinal axons much prefer to adhere to and grow on the surface of these large flattened cells than on the collagen substrate. Naturally we wondered whether the XR1 cells maintained the ability to support neurite outgrowth. We found that retinal axons grow freely over a confluent layer of XR1 cells whereas they do not do so over a line of kidney epithelial cells(A6). Moreover,we found that XR1 cells lay a factor onto the collagen substrate which remains after the cells are removed from the surface by hypoosmotic shock. This factor strongly enhances neurite outgrowth. A6 cells do not contribute such a factor. It is perhaps a little surprising that we have not been able to recover this activity from medium conditioned by XR1 cells, which leads us to suspect that it is membrane bound or secreted only at attachment sites. We have some reason to conclude that the factor in question may not be laminin. While it is true that using antisera to human and mouse laminin XR1 cells stain heavily and leave laminin impressions on culture dishes, so do A6 cells. Laminin purified from both human and mouse sources when attached to our culture dishes failed to promote neurite outgrowth, while the same treated dishes were proven effective in promoting neurite outgrowth, while the same treated dishes were proven effective in promoting neurite outgrowth from other neuronal types from rat CNS. Antiserum to human laminin which effectively blocked the outgrowth of chick ciliary ganglion neurons grown on human laminin treated dishes had minimal inhibitory effects on the outgrowth of Xenopus retinal axons grown on XR1 cell conditioned surfaces. Finally, while it is clear that the pial surface of the embryonic central nervous system contains a basement membrane which stains heavily for laminin and that the pioneering retinal ganglion cell axons grow in proximity to this layer of extracellular matrix in the developing optic tract, it seems equally clear at the light microscopic level that the growth cones of many of these neurons are not in intimate association with this basement membrane and are probably not in contact with it. For these reasons we speculate that XR1cells are providing a neurite promoting factor which is either highly species specific laminin or not laminin, and furthermore that in the Xenopus embryo laminin is not essential to the oriented outgrowth of retinal ganglion cell axons. Support by NIH.

221.13 ISOLATED SPINAL CORD NEURONS DIFFERENTIATE AND GROW IN EARLY HYDRATED COLLAGEN LATTICE CULTURE. J.R. Cochran* and P.W. Coates. Department of Anatomy, Texas Tech University HSC School of Medicine, Lubbock, Texas 79430.

Dissociated neurons from day 10-11 chick embryo spinal cord were obtained by a combination of gentle mechanical disruption and trypsinization. Low density hydrated collagen lattices (HCL) were cast in 35 mm tissue culture dishes and conditioned with Medium 199 with 1% antibiotic-antimycotic and 10% fetal calf serum. Cells were plated at $10^5$ per ml per dish, and incubated in a humidified incubator at 37° C (95% air + 5% $CO_2$). At the end of each of 3 days, cultures were fixed for morphological and quantitative evaluation. Within 24 hr, at least some neurons exhibited one long process, interpreted as being an axon, and one or more shorter, tapered and sometimes branched processes interpreted as being dendrites. Phase contrast and light microscopy of Nissl and silver stained HCL revealed multipolar and other neurons with morphologically differentiated features. Only single neurons, i.e., not in contact with other cells or cell processes, and with at least one process longer than the cell diameter, were analyzed statistically. A minimum of ten neurons were measured on each day of the test period for eight different experiments using a Bioquant II Image Analyzer in combination with a phase contrast microscope. Results were compared using ANOVA with Newman-Keuhl's Multiple Range Test. The mean length of axons and dendrites alone and combined as total new growth per neuron, and the mean number of branch points, segments and terminals all increased from 50 to over 250 percent ($P \leq 0.05$) from day 1 to day 3. There was also a trend for the number of primary processes to increase over the test period. These observations extend previous work showing enhanced neurite outgrowth from spinal cord explants in HCL culture (Coates, Anat.Rec.208:33A,1984). The present data show that isolated spinal cord neurons differentiate and grow in early HCL culture. (Partial support from the APA and Tarbox Institute.)

221.14 A SYNTHETIC TETRAPEPTIDE INHIBITS DORSAL ROOT GANGLION CELL ADHESION TO ONE, BUT NOT ANOTHER, CELL BINDING REGION OF FIBRONECTIN. S.L. Rogers, P.C. Letourneau*, L.T. Furcht and J.B. McCarthy*. Dept. of Anatomy, Univ. of Minnesota, Minneapolis, MN 55455.

The adhesive extracellular matrix (ECM) glycoprotein fibronectin (FN) appears to be involved in neural crest cell migration and differentiation, and supports neurite extension by peripheral neurons in vitro. Mechanisms of cell interaction with ECM components like FN can be studied using proteolytic fragments of the molecule, with the aim of identifying the domains of fibronectin that mediate cell attachment and defining relevant cell surface receptors. A 75Kd "cell binding" region (Hayashi and Yamada, J. Biol. Chem.,258:3332,1983) and heparin binding region (Rogers et al., J. Neurosci.,5:369,1985) mediate cell type-specific activities, including neurite extension. We have used a synthetic tetrapeptide (Arginyl-Glycyl-Aspartyl-Serine; purchased from Peninsula Laboratories) representing the "cell binding determinant" of FN (Pierschbacher and Ruoslahti, Nature,309:30, 1984) in competitive inhibition assays to distinguish dorsal root ganglion (DRG) cell adhesion to this determinant from adhesion to the 33Kd heparin binding cell attachment fragment. Tissue culture wells were treated with FN (20 and 5 ug/ml), the 75Kd fragment (molar equivalents of 100 and 20 ug/ml FN), the 33Kd fragment (molar equivalents of 100 and 20 ug/ml FN), laminin (20 ug/ml) or polyornithine (100 ug/ml). Dissociated embryonic chick DRG cells were cultured on these substrata in serum-free medium for 1-3 hours in the presence and absence of tetrapeptide ($10^{-3}$ M). Nonadherent cells were then removed by rinsing, and attached cells fixed with glutaraldehyde and quantified with an Optomax image analyzer. In the presence of tetrapeptide, cell attachment to FN was reduced by up to 60% relative to controls, with the greatest effect at low concentrations of FN. Inhibition of attachment to the 75Kd fragment reached 80% at low levels of the substratum-bound fragment. In contrast, attachment to the 33Kd fragment was never reduced more than 20% by the tetrapeptide, and it had relatively little (laminin) or no (polyornithine) effect on attachment to the other substrata. These observations support our hypothesis that DRG cells can interact with the two cell binding regions of FN via distinct surface properties. We are now investigating the role of the cell binding tetrapeptide in neurite extension on FN.

221.15 PROMOTION OF ADHESION AND NEURITE OUTGROWTH FROM RETINAL AND TECTAL EXPLANTS BY SCHWANN CELL CONDITIONED MEDIUM. L.K. McLoon. Dept. of Opthal., Univ. of Minn., Minneapolis, MN 55455.

The role of non-neuronal cells in the guidance or promotion of axonal outgrowth is poorly understood. Recent work has indicated that central nervous system tissue (CNS) grown on various non-neuronal monolayers in vitro results in preferential outgrowth of the neurites on glial as compared to non-glial cells (Fallon, 1985; J. Cell Biol.). We have examined the ability of Schwann cell conditioned medium to promote adherence and neurite outgrowth from embryonic retinae and tecta in explant culture. Schwann cell conditioned medium (SCCM) was obtained from cultures of newborn dorsal root ganglia by standard procedures (Salzer et al., 1980; J. Cell Biol.). Retinae and tecta were dissected from rat pups on embryonic day 14 and placed in explant culture on a variety of substrates. Explants were cultured on tissue culture plastic, collagen and polylysine substrates in supplemented MEM. A second set of explant cultures were set up on the same three substrates and SCCM was added. The retinal and tectal explants were examined for adherence and neurite outgrowth at 1, 2 and 5 days in vitro. Neither the retinal nor the tectal explants adhered well to the plastic or collagen coated substrates. Both retina and tectum adhered well to polylysine coated dishes. Minimal or no neurite outgrowth was seen from the retinal explants grown on a polylysine substrate. As early as 1 day in culture tectal explants grown on polylysine substrates showed rapid extension of neurites, which tended to be heavily fasciculated. The addition of SCCM to retinal explants resulted in an increase in the adhesion of the explants, even on to plastic and collagen substrates. There was also a rapid extension of neurites from these retinae. Although the outgrowth was mainly radial, many crooked fascicles were also seen. Tectal explants, however, did not respond to the SCCM with increased neurite outgrowth, nor did they show increased adherence to previously unfavorable substrates.

Thus it appears that there is a factor in Schwann cell conditioned medium that has the ability to promote neurite outgrowth from embryonic retina in vitro. (Supported by NIH grant EY05432 and the Minnesota Medical Foundation.)

221.16 PROTEINS ASSOCIATED WITH GROWTH OF RAT HINDBRAIN NEURONS IN VITRO S. Finklestein, N.I. Perrone-Bizzozero and L.I. Benowitz. Departments of Neurology and Psychiatry, Massachusetts General Hospital; and Mailman Research Center, McLean Hospital, Belmont, MA 02178

Using conditions that were either permissive or inhibitory to neurite outgrowth, we examined the relationship between outgrowth and the expression of specific proteins in vitro. Freshly-dissected hindbrain from E17 rat embryos was mechanically dissociated and plated in Minimum Essential Medium (Eagle's; with added glucose, glutamine, vitamins, and gentamycin) onto poly-L-lysine coated culture dishes at a density of 3 X $10^6$ cells/ml. Cultures were incubated at 37°C in a 5% $CO_2$/humidified air atmosphere for 48h. They also received either: (a) 5% fetal calf serum/5% heat-inactivated horse serum, which allowed neurite outgrowth; (b) no serum, which allowed cell survival but no outgrowth; or (c) serum plus tunicamycin, which prevented protein N-glycosylation as well as neurite outgrowth. Tunicamycin was either present for the full 48h, or only for the last hour before labeling. Cultures were labeled with 40 uCi of $^{35}S$-methionine (1000 Ci/mmole) in methionine-free medium for 1 hour, and then incubated for an hour in an excess of non-radioactive methionine. Cells were homogenized, and the particulate fraction subjected to 2-dimensional gel electrophoresis and fluourography.

Cultures grown under the various conditions showed striking differences in neurite outgrowth, as well as in overall protein synthesis and the synthesis of specific molecular species. Of particular interest was a highly acidic 48,000 dalton (48 kD) protein that was synthesized heavily only under conditions of active neurite outgrowth (Table). The properties of this protein resemble those of a rapidly-transported glycoprotein that appears in the regenerating goldfish optic nerve (Perrone-Bizzozero and Benowitz, these abstracts), and may also be similar to the 43 kD protein described in association with axonal growth in other developing and regenerating neural systems (J.H.P. Skene, Cell 37 :697, 1985). Positive identification is now being undertaken using enzyme cleavage methods.

| Culture Conditions | Total Radioactivity in Particulate Protein Fraction (cpm) | 48 kD Acidic Protein |
|---|---|---|
| Serum alone | 2 X $10^6$ | +++ |
| No serum | 2 X $10^5$ | +/- |
| Serum + tunicamycin (48h) | 2 X $10^5$ | - |
| Serum + tunicamycin (1h) | 1 X $10^6$ | - |

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221.17 NEURITE GROWTH FROM EARLY NEURAL TUBE EXPLANTS IN DEFINED AND SERUM-SUPPLEMENTED MEDIA. D.B.Wayne* and M.B.Heaton. Dep't. of Neuroscience, Univ. of Fla. Coll. of Med., Gainesville, FL 32610.

The trigeminal region of chick metencephalic neural tube was explanted into culture at stage 10-12 (40-45 hours). This early date preceeds most neuronal differentiation in that region. Culture at this time must support the continued differentiation of the neurons within the explant. This differentiation includes the initiation of neurites rather than the early regenerative events observed with tissue excised at later developmental times. The growth observed in defined supplements as opposed to serum-supplemented medium differed both quantitatively and qualitatively. The explants were plated on a combined collagen and polyornithine (coll/PORN) substratum. A basic medium of Ham's F-12 with glutamine, penicillin/streptomycin, and fungizone was supplemented with either 11 ml.of fetal calf serum or the following constituents: progesterone (20nM), putrescine (100$\mu$M), insulin (o.5 mg/100ml), transferrin (o.5 mg/100ml), sodium selenite (o.5$\mu$g/100ml), bovine serum albumin (50 mg/100ml) and a potassium concentration adjusted to 20 mM. Camera lucida drawings of the explants were made after 4, 8 and 12 days in culture. Neurite outgrowth was quantitated by counting the numbers of neurites which intersect concentric circles of an overlying template. Outgrowth is expressed as a percent of the total outgrowth possible, that is, relative to the maximum outgrowth demonstrated within that group. The defined medium groups consisitently demonstrated a greater percentage of outgrowth at each age. The averaged growth was at day 4 (n=16), serum=11.2%, defined=64.8%; at day 8 (n=16), serum=6.7%, defined=43.8%; and at day 12 (n=8), serum=6.9%, defined=44.3%. In both conditions, most neurite growth was confined to the mat of primarily non-neuronal cells which surround the explant core. The non-neuronal cells formed a denser and more closely packed cell mat in the serum-supplemented condition. Neurite growth cones extended off the cellular mat onto the coll/PORN substratum more frequently in the defined than in the serum-supplemented condition. In defined medium, those neurites which grew onto the exposed substratum extended further from the explant periphery than did those in serum-supplemented medium. In the defined medium employed, metencephalic explants show a healthy outgrowth of neurites, thus indicating that the differentiation of some brainstem neurons is not dependent on the presence of serum in culture and, in fact, may be hindered in some way by the presence of serum.

(Supported by NIH grant NS20387 to M.B.Heaton.)

221.18 AN ANTIBODY WHICH BLOCKS OUTGROWTH OF SUBPOPULATION OF NEURITES. M. Yamamoto, A.M. Boyer*, H. Tanaka and J.N. Wood*. Dept. of Biochem. & Neuropath., E.K. Shriver Ctr., Waltham, MA 02254.

Guidance of axonal outgrowth is an important problem to understand the highly organized neuronal connections between specific neuronal cell groups. A monoclonal antibody, 4D7, raised against embryonic rat forebrain, recognized polypeptide antigens which might play a role for proper axonal growth of subpopulation of neurons.

Immunocytochemical study with 4D7 was carried out on developing (E10-P14) and adult mouse brain. From E10-E17, the selective population of axons was labeled, which contrasted sharply with the staining of an anti-neurofilament antibody (C2). At E10, motoneurons and their axons were the only 4D7 immunoreactive population in entire nervous system. At this stage, however, C2 already showed many more groups of fibers, for example, neurites from the dorsal root ganglion (DRG). At E12-13, axons from DRG, trigeminal ganglion, motoneurons, VIIIth, VIIth, IInd cranial nerves, commisural fibers in the ventral part of the hindbrain, fasciculus retroflexus (FR), fibers running along the dorsal surface of the cerebellum were positive. At E14-15, staining was only seen in the cortex, FR and optic tract. In the cortex, 4D7 immunoreactivity is associated only with the intermediate zone (IZ). The staining in the IZ became thinner at E17 and at P0, only weak staining remained in cortical white matter. From P0-P14, the juxtaposition of the external granule cell layer and the molecular layer in the cerebellum was the only region which was immunoreactive with 4D7. In adult, no staining was observed.

The tissue culture prepared from E13-15 rat brainstem and grown in serum free medium, revealed the immunoreactivity on the plasma membrane of a subpopulation of neuronal processes. 4D7 positive cells were distinct from GFAP positive cells. Addition of 4D7 antibody (purified IgM, 20ug/2ml of media) in the same culture blocked neurite outgrowth of 4D7 immunoreactive cells. They showed only short stubby neurites. The other neurites which did not show 4D7 immunoreactivity showed no effect. Control cultures treated with another IgM anti-mouse antibody did not show any significant change on 4D7 positive neurites. This effect of 4D7 antibody was observed on cultures plated on various substrates (poly-L-lysine, fibronectin, collagen and laminin).

The 4D7 antibody recognized two immunoreactive protein bands (110kD & 140kD) by Western blot on homogenate of E15 mouse brain.

Thus, the 4D7 antigens, which are expressed on a subpopulation of developing axons in a short period of time might play a crucial role for axonal growth. Supported by NIH grant HDO-5515-15.

222.1 EFFECT OF DIFFERENT CARBOHYDRATES ON SYMPATHETIC ACTIVITY IN HEART AND INTERSCAPULAR BROWN ADIPOSE TISSUE (IBAT) OF THE RAT. M.C. Walgren , J.B. Young*and L. Landsberg*. Dept of Medicine, Beth Israel Hospital and Harvard Medical School, Boston, MA 02215.

Sucrose ingestion increases sympathetic nervous system (SNS) activity, an effect which may contribute to the thermogenic response to carbohydrate (CHO). Because different CHOs stimulate insulin secretion to a variable extent and insulin is a potential link between dietary CHO and SNS activation, the possibility that CHOs might differ in their potency as sympathetic stimulants was examined in the following studies. Norepinephrine turnover (NETR), a measure of SNS activity in tissues of experimental animals, was measured in rats (CD males, 150-175g, Charles River) fed a lab chow ration supplemented (to increase caloric intake by 50%) with corn starch (CS), sucrose (S) or fructose (F) for a 10 day period. These studies also contained a control group (Ch) which was fed a chow ration (8g/100g BW) equal to that given rats consuming the supplemented diets. Compared to Ch-fed rats, NETR in both heart and IBAT was increased in animals receiving supplemental CS or F. Small differences between isocaloric supplements of CS and S and between F and S in stimulating NETR in either heart or IBAT, though suggested in several experiments, were not consistently present. In one study, NETR in heart increased from 24.0ng/h in Ch to 33.1 in S ($p<.05$ vs Ch) and to 50.1 in CS ($p<.05$ vs Ch), and in IBAT NETR increased from 11.6ng/h in Ch to 17.4 in S ($p<.05$ vs Ch) and to 30.3 in CS ($p<.05$ vs Ch). F and S augmented cardiac NETR from 21.4ng/h to 46.9 ($p<.05$ vs Ch) and 55.6 ($p<.05$ vs Ch), respectively. In addition, the possibility that saccharin, which may slightly increase insulin secretion, might also stimulate NETR was examined. Five days of feeding a saccharin-supplemented chow diet (0.2% by wt, estimated to be comparable in sweetness to a 50% sucrose supplement) did not alter NETR in either heart or IBAT compared to that observed in Ch-fed rats (13.9ng/h in Ch, 13.5 with saccharin, $p>.05$). In summary, a) CHO-induced stimulation of SNS activity in heart and IBAT occurs in response to ingestion of CHOs other than sucrose and is not appreciably affected by the type of CHO consumed; and b) the sweet taste of CHO-containing food does not appear to influence the SNS response to CHO. These data indicate that if insulin is an important link between dietary CHO and SNS activity, then the SNS response to CHO is not sensitive to differences in insulin secretion observed following ingestion of various CHOs.

222.2 SYMPATHETIC AFFERENT AND PREGANGLIONIC NEURONS LABELLED BY HORSERADISH PEROXIDASE APPLIED TO THE HYPOGASTRIC NERVE AND THE SACRAL SYMPATHETIC CHAIN OF THE RAT. I. Nadelhaft and K.E. McKenna. VAMC and University of Pittsburgh Med. Sch., Depts. of Neurological Surgery and Pharmacology, Pittsburgh, PA 15240.

The major afferent and efferent innervation of the urinary bladder and other pelvic viscera comes, via the pelvic nerve, from neurons in the L6-S1 cord and dorsal root ganglia (DRG) [ Nadelhaft and Booth JCN 226, 238 (1984)]. The sympathetic innervation of these areas arises in the upper lumbar cord and the axons are located in the hypogastric nerve and the sympathetic chain. Horseradish peroxidase (HRP) was applied to either the cut hypogastric nerve (unilateral) or the sympathetic chain (bilateral, between the L6 and S1 sympathetic chain ganglia). Animals were sacrificed 2 days later and sections processed with tetramethylbenzidine as the chromagen for HRP. Hypogastric afferents were found bilaterally in smaller DRG neurons from T13 to L3 (90% in L1-L2). Doubling the numbers from the hypogastric experiment to compare with the results from the sympathetic chain experiment, resulted in an average of 200 sensory neurons. Sympathetic chain afferents were located in DRG from T11 to L2 (90% in T13-L2). A total of 200 neurons were found. By comparison, HRP applied to the pelvic nerves labelled an average of 3000 neurons in L5-S2 DRG (95% in L6-S1) and none were found elsewhere.

Hypogastric preganglionic neurons were located bilaterally in spinal cord segments L1-L3, with the majority (75%) in L2. Within the segments, the majority (70%) were found in the dorsal gray commissure and the remainder at the intermediolateral edges of the gray matter. Sympathetic chain preganglionic neurons were located in spinal cord segments T11 to L2 with the majority (78%) in L1-L2. Within the segments, the majority (90%) were found at the intermediolateral edges of the gray matter with the balance distributed in the intermediate gray and the dorsal gray commissure. There were no neurons found in the sympathetic cord when HRP was applied to either the pelvic or pudendal nerves.

In summary: 1) Sympathetic afferents innervate the pelvic viscera via two pathways: the hypogastric nerve and the sympathetic chain. There are approximately one sixth as many sympathetic afferents as there are parasympathetic afferents. 2) The spinal cord distribution of sympathetic preganglionic neurons sending axons into the hypogastric nerve is strikingly different from those sending axons into the sympathetic chain. The former are mainly located medially whereas the latter are located laterally. Perhaps this organizational difference is reflected in a functional difference (as yet undetermined).

222.3 EVIDENCE FOR LOCAL NEURAL MODULATION OF SYMPATHETIC INFLUENCES ON THE FELINE BLADDER. D.C. Rudy* and J.W. Downie. Depts. of Pharmacology and Urology, Dalhousie University, Halifax, Nova Scotia B3H 4H7

The bladder is sympathetically innervated by the hypogastric nerves and by the sympathetic chain via the pelvic nerves. Both of these sympathetic supplies produce a brief contraction (volume decrease) followed by a more prolonged dilation (volume increase) in the bladder. Our initial studies showed that the dilatory component of the response to sympathetic stimulation is enhanced as intravesical pressure (IVP) is increased and this relationship persists in decentralized bladder preparations. Herein we describe our studies of this phenomenon. In chloralose-anesthetized cats the bladder was decentralized by section of sacral spinal roots, both hypogastric nerves (HGN) and one sympathetic chain (SC). The IVP was controlled by a reservoir with large surface area via a bladder cannula. HGN and SC stimulation evoked similar contraction-dilation responses in the bladder although both phases were usually larger for HGN stimulation. Raising IVP from 5 to ≥10 cmH20 increased the proportion of cats showing the dilation component from 40% to 82% for HGN stimulation, and from 46% to 65% for SC stimulation. As well, the magnitude of the relaxation response was similarly increased in 62% with HGN stimulation and in 42% with SC stimulation. Effects of increasing IVP were more reliable in later experiments where IVP was increased to ≥20 cmH20. In a separate group of cats the dilation from sympathetic nerve stimulation was compared to that produced by a maximal dose (5 µg ia) of isoprenaline (IP). At 5 cmH20 (low IVP), IP produced a significantly greater bladder dilation than either HGN or SC stimulation. At 20-30 cmH20(high IVP), the IP-induced response was larger but the HGN now produced an equivalent response. The ratios of dilation responses at high vs. low IVP were IP=2, SC=7 and HGN=28. Assuming that IP acts on smooth muscle only, the ratio of >1 indicates that end organ factors can contribute to enhancement of responses at high IVP. However, the much larger ratios seen for the /mpathetic nerve-mediated dilations indicates that end organ factors are not sufficient to account for these changes. Furthermore, in several cats there was no dilatory response to sympathetic stimulation at low IVP despite a clear IP response. The equivalence of responses at high IVP for both HGN stimulation and IP rules out the argument that a difference in agonist concentration plays a role. We therefore conclude that the increased bladder distension produced by raising IVP in this preparation activates a local mechanism that modulates the sympathetic neural input to the bladder. This modulation is exerted mainly at a level other than the end organ and therefore is likely to be on the nerves themselves. The nature of the modulatory influence is unclear but may be a humoral substance (e.g. prostaglandin) or a "local reflex" arc. (M.R.C. and A.U.A. supported.)

222.4 EFFECTS OF OVARIAN HORMONES ON AUTONOMIC RESPONSE OF RAT'S URINARY BLADDER TO CARBACHOL. H. R. Amouzadeh* and S. Sangiah. Dept. of Physiol. Sci., Oklahoma State Univ., Coll. of Vet. Med., Stillwater, OK 74078

Estrogen and progestrone have been extensively investigated for their role in modulating the mechanical activities of various smooth muscles. It has been shown that estrogen potentiates the in vitro autonomic response of rabbit's urinary bladder and guinea pig's gallbladder. Progestrone, however, has been shown to have an inhibitory effect on guinea pig's gallbladder. Adult male albino Sprague-Dawley rats weighing between 350 to 500g were divided into three groups. Group one (n=15) received subcutaneous (SC) injection of peanut oil for 6 to 9 days. Group two (n=10) and group three (n=6) were treated with SC injection of 17-β-estradiol (400 µg/kg) and progestrone (2mg/kg) in peanut oil for 9 and 6 days respectively. Detrusor muscle strips of uniform size (6mm x 2mm) from the bladder of each rat were cut under light anesthesia and suspended in isolated organ bath (20 ml). The contractile response of these strips to various doses of carbachol (0.5-100 µM) were recorded using a physiograph. 17-β-estradiol treatment shifted the dose response curve to the left while progestrone treatment shifted it to the right. These results are consistent with the previous reports and demonstrate the modulatory effect of ovarian hormones on contractile response of rat's urinary bladder. Mechanism(s) of these effects are currently being investigated.

222.5 **TRACER DIFFUSION FROM INJECTION SITES DISTORTS ORGAN INNERVATION MAPS: LIMITING DIFFUSION PREVENTS LABELING OF VAGAL PREGANGLIONIC NEURONS AFTER TRUE BLUE INJECTIONS IN THE PANCREAS** E.A. Fox* and T.L. Powley. Lab. of Regulatory Psychobiology, Purdue Univ., West Lafayette, IN 47907.

Although visceral mapping studies have typically provided minimal or no controls for tracer leakage, diffusion from injection sites is probable on the basis of several published observations: 1) the serosal and mesenteric membranes which surround the viscera are permeable to HRP and other small molecules, 2) IP injections of HRP and True Blue (TB) result in significant labeling of dorsal motor nucleus of the vagus (DMN) neurons, 3) physical isolation of an HRP-injected organ can decrease the DMN cell label as well as prevent spinal cord label, and 4) nerve soaks with tracers generally result in greater localization and fewer labeled cells than organ injections. Such observations suggest that viscerotopic maps based on tracer injections may be distorted or exaggerated because of spread of tracer and subsequent uptake by terminals in other tissues. In the present experiment, the effects of a diffusion barrier on the pattern of cell labeling after tracer injections in the pancreas were examined.

Male Sprague Dawley rats received two 7.5µl injections of 10% TB in the splenic lobe of the pancreas (n=4). These TB injections labeled cells in the DMN, nucleus ambiguus (NA), and celiac ganglia (CG), replicating the results of earlier experiments. In other groups of animals receiving the same TB injection, a barrier formed from a plastic wound spray (pyroxylin solution, New Skin) was applied to the surfaces of either the stomach and adjacent intestines (n=4) or the splenic pancreas (n=8). Just prior to sacrifice (72-73h after TB injections), 6 of the latter rats and an additional 5 control animals (TB alone) were tested for insulin secretion in response to right (RCV) and then bilateral (BCV) cervical vagus stimulation. Placing the barrier either on the viscera adjacent to the pancreas or on the pancreas itself prevented virtually all labeling of DMN and NA cells and reduced the labeled CG cells by 50-70%. RCV and BCV stimulation produced comparable increases in insulin secretion in animals with and without the barrier.

The combined observations of normal insulin secretion and intact labeling of many CG cells after barrier application indicates that the innervation of the pancreas was still functional and suggests that the barrier did not reduce cell labeling by neurotoxic effects. Thus, the failure to label vagal preganglionics when tracer diffusion from the pancreas injection site was limited suggests that the number of DMN parasympathetic preganglionics innervating the pancreas may have been significantly overestimated in previous tracer experiments. Further, diffusion may complicate the interpretations of cell labeling after injections in other viscera. (USPHS grant AM27627 and David Ross Fellowship)

222.6 DISTRIBUTION AND ORIGIN OF CALCITONIN-GENE RELATED PEPTIDE IMMUNOREACTIVE FIBERS INNERVATING THE PANCREAS IN THE RAT. C.Sternini, K.Anderson* and N.Brecha. Center for Ulcer Research and Education and UCLA School of Medicine, VA Medical Center Wadsworth, LA, CA 90073.

Calcitonin-gene related peptide immunoreactivity (CGRP-I) has been reported in the nervous and endocrine systems. Using new CGRP antisera raised in rabbits against [Tyr]-rat CGRP(23-37) coupled to keyhole limpet hemocyanin via glutaraldehyde we studied the distribution of CGRP-I in the rat pancreas and the possible origin of the CGRP immunoreactive fibers innervating this organ. Normal, capsaicin- and 6-hydroxydopamine (6-OHDA)- treated and vagally denervated rats were perfused with a paraformaldehyde solution. In some rats the fluorescent retrograde tracer Fast Blue (FB) was injected into the pancreas, placed on the pancreatic surface or dropped into the abdominal cavity. Pancreata and the structures which are presumed to give rise to the fibers innervating the pancreas were removed and sectioned. Tissue sections were processed by the indirect immunofluorescence technique. Specificity was determined by incubating sections with preimmune serum or primary antiserum preabsorbed with 10uM rat CGRP. In the exocrine pancreas CGRP-I is localized to varicose processes distributed among the acini, in the intralobular and interlobular connective tissue and to the duct system as well as to scattered cells disseminated among the acini. In the endocrine pancreas thin immunoreactive processes are present around and throughout the islets and single or clustered labeled cells are distributed mainly at the periphery of the islets. Particularly numerous fibers are associated with blood vessels. In rats treated neonatally with the small diameter sensory neurotoxin capsaicin there was a marked reduction or a complete elimination of CGRP immmunoreactive fibers innervating the pancreas and blood vessels. Treatment with the sympathetic neurotoxin 6-OHDA and subdiaphragmatic vagotomy did not affect the immunoreactive staining. CGRP containing cell bodies are colocalized with most of the FB labeled cells in the dorsal root ganglia, but only with few FB positive cells in the nodose ganglia. No CGRP positive cells were observed in the coeliac ganglion. In the brainstem some somata of the rostral part the nucleus ambiguus contain both FB and CGRP-I. These results demonstrate that 1)CGRP-I in the pancreas is localized to endocrine-/paracrine-like cells and to nerve fibers, 2)CGRP immunoreactive fibers originate from extrinsic sensory sources and 3)CGRP containing neurons which give rise to fibers innervating subdiaphragmatic viscera are mainly located in spinal dorsal root ganglia. These observations suggest that CGRP may have both an endocrine and neurotransmitter role in the control of pancreatic functions.

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222.7 HISTOCHEMICAL EVALUATION OF THE ACTIVITY OF SINGLE ENTERIC NEURONS: VERIFICATION OF THE CYTOCHROME OXIDASE TECHNIQUE. G.M. Mawe and M.D. Gershon. Anatomy and Cell Biology, Columbia University, Coll. of P&S, New York, NY 10032.

Wong-Riley and others have established that the histochemical activity of the mitochondrial enzyme, cytochrome oxidase (C.O.), reflects the metabolic activity of CNS neurons. Experiments were done to determine whether the C.O. activity of enteric neurons could be acutely influenced by pharmacologically opening and closing voltage-dependent $Na^+$ channels in vitro, a finding that would indicate that the technique reflects neuronal metabolism in gut as well as brain. Dissected preparations of guinea pig myenteric plexus were incubated in oxygenated krebs solution ($37^{o}$) for 8 hours. Experimental groups contained veratridine (0.1uM - 0.1mM), tetrodotoxin (TTX; 0.5uM) and veratridine + TTX. C.O. activity was demonstrated by incubating formaldehyde (4%)-fixed tissue with cytochrome C (0.3g/l) and diaminobenzidine (0.5g/l) for 1 hour. Intensity of C.O. activity was evaluated by counting numbers of active cells/ganglion area and by densitometrically measuring light transmission through the C.O. reaction product. In control preparations, there were 100±3 neurons/ganglion, of which 48±3 neurons/ganglion showed significant C.O. reaction product. This indicates that C.O. activity is not uniform among enteric neurons. The numbers of C.O.-active cells per unit of ganglionic area, and the density of reaction product within labelled neurons, was increased significantly by veratridine at all concentrations examined ($p<0.001$), however, values for tissue treated with TTX or veratridine + TTX were not significantly different from the controls. Intracellular electrophysiological experiments indicated that at 1uM veratridine caused myenteric neurons to fire intermitent bursts of spontaneous action potentials but to maintain their resting potential. Higher concentrations of veratridine (>5uM) caused membrane depolarization and prevent neuronal discharge. The degree of depolarization was concentration-dependent. Maximal stimulation of histochemically determined C.O. activity by veratridine occurred at 1.0um. Less stimulation was seen at 5uM but C.O. activity thereupon increased again as the concentration of veratridine was raised (to 0.1mM). These data indicate that C.O. activity probably does provide a histochemical reflection of the metabolic activity of myenteric neurons. It is likely that the initial intense stimulation of C.O. activity at a low concentration of veratridine is due to repetitive discharge of action potentials. The later activation of the enzyme may be related to attempts by depolarized neurons to pump out $Na^+$ that veratridine caused to accumulate in the cells. The C.O. histochemical technique should thus be very useful in identifying which enteric neurons are active in the control of intestinal activity. Supported by grants NS12969 and NS07062.

222.8 **AN EM ANALYSIS OF THE FIBER COMPOSITION OF THE HEPATIC BRANCH OF THE VAGUS.** J.C. Prechtl* and T.L. Powley, (SPON: W. Pak) Lab. of Regulatory Psychobiology, Purdue University, West Lafayette IN 47907.

The hepatic branch of the abdominal vagus has been the subject of an extensive series of physiological analyses. Although this research is complemented by LM descriptions of the nerve (e.g. Prechtl & Powley, JCN, in press: rat; Kemp, Aust. N. Z. J. Surg., 1973: dog), an EM analysis of its fiber content is lacking and its projection through the lesser omentum has not been fully described. We have done an analysis of the fine structure of the hepatic branch in several animals taken from a larger series prepared for an ultrastructural characterization of the abdominal vagus.

The abdominal vagi of six adult male Sprague Dawley rats (Hormone Assay, Chicago) were prepared for electron microscopy. From each specimen a complete montage (X10,000) of the hepatic was assembled and all the fibers were counted. To photograph the hepatic branch as a coherent bundle we sampled it immediately (<130 µ) after it separated from the anterior vagus (n=5); one sample was taken slightly more distally. For LM analysis, we also took broad cross-sections of the hepatic branch within the lesser omentum approximately halfway between the esophagus and hepatic artery, at a site where the nerve is often surgically and/or electrophysiologically manipulated. Supplementary data on the branching patterns were also obtained from osmicated whole mounts.

The hepatic branch contained 2887±287 unmyelinated fibers and relatively few (21±4) myelinated fibers. However, half (54±5%) of these myelinated fibers were clustered together. Converging evidence from whole mounts and the montages indicate that these myelinated fibers formed a subpopulation that was already segregated on the medial side of the anterior vagus, initially remained aggregated in the medial side of the hepatic branch, and then formed a coherent small fascicle which separated from the hepatic early in its course. Soon after the hepatic branch began its rostromedial trajectory in the lesser omentum parallel with the hepato-esophageal artery, it contained an endoneurial paraganglion (cf. also Prechtl and Powley, in press). The EM specimen in this series was not unlike paraganglia which have been reported in other autonomic nerves; it was well vascularized and contained numerous cells with dense-cored vesicles. Further distally, the hepatic branch formed a plexus of 3-6 bundles which subsequently recombined to form 3 or 4 major bundles (40-80 µ in diameter). Also conspicuous at this segment were 2-3 small fascicles (<20 µ in diameter). In addition to the hepato-esophageal artery and vein accompanying the hepatic bundles, a lymphatic vessel was also a regular feature. The volume of the lymphatic was greater than that of the vein and artery combined.

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222.9 **DIFFERENT FREQUENCY- AND TEMPORAL-RESPONSE PROFILES FOR ENDOCRINE PANCREATIC HORMONES, GASTRIC ACID, AND CARDIOVASCULAR PARAMETERS USING CERVICAL VAGAL STIMULATION IN THE ANESTHETIZED RAT.** H.R. Berthoud and T.L. Powley, Lab. of Regulatory Psychobiology, Purdue University, West Lafayette IN 47907.

The viscerotopic and functional organization of the vagal preganglionic motor neurons is not well defined. Since previous research has demonstrated that efferents innervating the abdomen include subpopulations with different soma sizes and different axon diameters and, further,since it is well established that cardiovascular preganglionics consist predominantly of large neurons with B-fiber processes, we examined the possibility that different functional pools of vagal efferents to the abdomen might have different physiological properties. The experiment determined the frequency-response and the temporal-response profiles of one gastric (acid secretion), two pancreatic (insulin and glucagon secretion) and two cardiovascular (mean arterial blood pressure and heart rate) responses.

Either the right or left peripheral end of the cut cervical vagus was stimulated with constant intensity (1mA) and pulse duration (1msec), and variable frequency (pps) or train duration in 12 h food-deprived, urethane/chloralose anesthetized, adult male Sprague Dawley rats. Potential sympathetic responses resulting from surgical trauma, anesthesia or stimulation were suppressed by continuous infusion with α- and β-blockers. Blood was sampled continuously using a method of simultaneous transfusion of whole donor blood. Gastric acid was measured on-line with a continuous gastric irrigation procedure.

Stimulation with 10 min trains of 1,2,4,8 and 16 pps resulted in 3 distinct response profiles: Gastric acid output was half-maximal at below 1 pps, a frequency at which both of the pancreatic responses and both of the cardiovascular responses were less than 10% of their maximal values, and peaked at 2 - 4 pps. Plasma insulin and glucagon responses were half-maximal at 3 - 4 pps, and the cardiovascular responses reached half-maximal at well above 8 pps. These characteristic response profiles were similar for both right and left cervical vagal stimulation. Prolonged stimulation at 4pps for 60 min produced distinctly different temporal response profiles as well. In addition to confirming the accepted view that vagal motor units supplying the heart are fast-conducting large-caliber fibers, the present data suggest the possibility that gastric and pancreatic efferents in the rat consist of two different classes of neurons, a pool with predominantly fine C-fibers innervating the gastric parietal cells and a pool with somewhat larger C-fibers innervating the endocrine pancreas.

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222.10 AFFERENTS TO THE NUCLEUS TRACTUS SOLITARIUS-DORSAL MOTOR NUCLEUS OF THE VAGUS IN THE PIGEON. M.L. BERK. Dept. of Anatomy, Marshall Univ. Sch. of Med., Huntington, WV 25704.

The nucleus tractus solitarius(NTS) and the dorsal motor nucleus of the vagus(DMN) of the pigeon are composed of cytoarchitectonically distinct subnuclei, which have organ specific connections (Katz and Karten, '83). This study represents the initial phase of a project to determine the central neural connections of NTS-DMN which could modulate the functions of specific organs. Afferents to NTS-DMN were determined by stereotaxically placed iontophoretic injections of either 25% horseradish peroxidase or 2% wheat germ agglutinin-horseradish peroxidase(WGA-HRP) into various rostral-caudal levels of the dorsomedial medulla. After a two day survival, the pigeons were perfused, brains sectioned, and tissue reacted with tetramethylbenzidine.

Retrogradely labeled perikarya were found in the forebrain at the following four loci: 1) many labeled soma adjacent to the ventrolateral border of the lateral ventricle of the ipsilateral telencephalon (stereotaxic level A10 to A7.25; labeled cell group bordered by n. accumbens), 2) numerous labeled cells in n. periventricularis magnocellularis (PVM) of the hypothalamus (bilateral representation), 3) a well-defined group of large, multipolar neurons in the stratum cellulare externum (SCE) of the lateral hypothalamus (stereotaxic level A6.00; unilateral representation), and 4) a few labeled parvocellular neurons in the medial preoptic area. The telencephalic cell group may be comparable to the mammalian bed nucleus of the stria terminalis. In the brainstem, contralateral NTS soma are retrogradely labeled. Additional brainstem input to NTS-DMN requires further study.

The specific subnuclei of NTS-DMN that are innervated by PVM of the hypothalamus have been previously demonstrated (Berk and Finkelstein, '83). In the present study, the projection of SCE neurons of the lateral hypothalamus to NTS-DMN subnuclei were investigated by anterograde transport techniques. Projections were observed to particular NTS subnuclei which include medialis superficialis, lateralis dorsalis, and medialis ventralis. Labeled fibers only project to the DMN subnucleus ventralis parvocellularis. The pattern of SCE innervation of NTS-DMN subnuclei is similar to PVM input except SCE fibers are fewer in number. The peripheral visceral connections of these NTS-DMN subnuclei (Katz and Karten, '83) suggest that the hypothalamus could modulate the functions of abdominal viscera as well as information from the aortic nerve.

In conclusion, medial and lateral hypothalamus and a telencephalic cell group have major inputs to NTS-DMN. The specific NTS-DMN subnuclei innervated by the telencephalic cell group awaits investigation. (Supported by the NIH Grant NS20512 and a grant from AHA, W.Va. affiliate)

222.11 **A QUANTITATIVE ASSESSMENT OF THE ROLE OF THE DORSAL MOTOR NUCLEUS OF THE VAGUS IN CONTROLLING GASTRIC ACID SECRETION.** W.B. Laughton* and T.L. Powley (Spon: G. S. Wasserman). Lab. of Regulatory Psychobiology, Purdue University, West Lafayette IN 47907.

Anatomical studies have implicated the dorsal motor nucleus of the vagus (DMV) as an important source of vagal preganglionic fibers to the abdomen (e.g. Kalia & Mesulam JCN,193:467; 1980). Data from physiological experiments have indicated that vagally mediated responses can be activated by stimulation of the dorsal medulla, however, these largely qualitative studies do not provide independent evidence that the DMV per se is the anatomical substrate effectively being stimulated in these studies, nor do they allow any inferences to be drawn concerning possible localization of function within the DMV.

Male Sprague-Dawley rats bearing pre-implanted gastric fistulae were food deprived overnight, anesthetized, and equipped with arterial and venous catheters. α- and β-blockers were administered. Semi-microelectrodes were introduced into the brainstem, and electrical stimulation (50μA, 50Hz, 1msec, 10 min) was carried out, with concomitant on-line monitoring of gastric acid secretion. Two-hundred and five electrode sites in 147 animals were located throughout the medulla. Sites located within the DMV and extending throughout its rostrocaudal extent yielded the strongest responses. Consistent with this conclusion, a "centroid" for activation of gastric acid was calculated by weighting each electrode placement proportionately to the magnitude of the gastric acid response resulting from its stimulation. These values were then entered into center-of-mass equations, allowing calculation of a weighted mean electrode location. This site was within the DMV, at approximately the mid rostro-caudal extent of the nucleus (i.e. AP level 6.52, 0.68mm lateral on Palkovits and Jacobowits (1974) atlas). That sites effective in eliciting gastric acid secretion did in fact tend to cluster around this derived centroid point was indicated by a significant negative correlation (r=-0.38, p<.001) between a)the magnitude of the acid response and b)the distance between the centroid point and the electrode location, and by the regression equation: A=19.1-8.3*D (A=acid response; D=distance), indicating that points farther from the centroid (in three dimensional space) were less effective than more proximal points. The magnitude of the acid response fell off less sharply as electrode location varied in the rostrocaudal dimension than in the coronal plane, consistent with the columnar shape of the nucleus.

No evidence was found for a differential representation of gastric secretomotor fibers along the rostrocaudal extent of the DMV as had been implied by some anatomical data, nor was there evidence for a left-right assymetry of function.

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222.12 BRAINSTEM NEURONAL RESPONSE TO AUTONOMIC MECHANISMS GOVERNING GASTRIC WALL TENSION. W.D. Barber and T.E. Burks, Departments of Anatomy and Pharmacology, College of Medicine, University of Arizona, Tucson, Arizona 85724.

This study has characterized a novel population of neurons in the solitary system (nucleus and tractus solitarius) in the brainstem of the cat which responded to moment-to-moment changes in gastric activity. The response of these neurons has provided further insight into central autonomic mechanisms which regulate visceral activity in this region of the medulla oblongata. Electrophysiological studies by others have reported that vagal fibers convey information from "in-series" receptors in the gastric wall which respond to both passive distention and active contraction of the stomach. Our study has shown that neurons in the solitary region, which responded to changes in gastric wall tension, had thresholds ranging from nondistended levels of intragastric pressure up to 10 cm of water. The local gastric intraarterial injection of substances which altered wall tension was accompanied by changes in the activity of these units. The relationship of unit activity and gastric wall tension was evaluated before and after the local intraarterial administration of atropine (10 ug/kg) at levels of intragastric pressure ranging from nondistended levels to 15 cm of water. Following atropine there was a decrease in unit discharge rate ranging from 32.08% ± 5.78 to 34.76% ± 9.17 when compared to pretrial values at the same level of intragastric pressure. This suggests that the discharge of these brainstem units reflects an unloading of the "in-series" receptors in the stomach wall due to the action of atropine upon the neuroeffector junction. The functional significance of this response suggests that it may play a role in receptive relaxation of the stomach during intake of food or drink to accommodate the ingested volume. In addition, unit activity related to the cardiovascular and respiratory systems was commonly observed in the subnuclei of the solitary system where these gastric responses were recorded. The neuronal connections and interactions among cell populations residing in the solitary complex suggests that this region may be an important site in the integration of peripheral and central influences which mold and shape visceral activity. (Supported by USPHS Grant AM31804)

222.13 INFLUENCE OF PERIPHERAL VS. CENTRAL (NUCLEUS TRACTUS SOLITARIUS) BOMBESIN ON GASTRIC PRESSURE IN THE RAT. S.E. Spencer, W.T. Talman. Lab. of Neurobiology, Univ. of Iowa and Vets. Admin. Med. Center, Iowa City, IA 52242.

Microinjections of the tetradecapeptide, bombesin (BOM), into the nucleus tractus solitarius (NTS) increases gastric tone. Tonic gastric pressure also increases when BOM is delivered intravenously (i.v.). We have, therefore, sought to determine if BOM acts both centrally and peripherally to modulate gastric pressures (GP). Thirty adult male rats were anesthetized with alpha-chloralose and instrumented for recording intra-arterial pressure, GP, and for the delivery of i.v. drugs. Each rat was placed in a stereotaxic frame, paralyzed, and ventilated via a tracheostomy tube. The dorsal surface of the brain stem was exposed and microinjections (25 nl) of BOM or saline vehicle were made through glass micropipettes stereotaxically placed into the dorsomedial NTS. Injection sites were marked with pontamine blue and confirmed histologically. In some experiments the spinal cord was transected at C1 and the vagi were transected bilaterally in the neck. BOM was delivered i.v. in 0.1 ml saline followed by a 0.1 ml heparinized saline flush. Alterations in tonic and phasic GP were statistically analyzed and expressed as mean ± standard error of the mean. BOM, but not vehicle, microinjected into the NTS or given i.v. elicited dose dependent increases in tonic GP. The central, but not peripheral, injection of BOM significantly increased the amplitude of phasic gastric waves. With injections into the NTS, the threshold dose for changing phasic GP was 7.8 fmoles, while the threshold dose for changing tonic GP was 7.8 pmoles. In contrast, tonic, but not phasic, GP increased after the i.v. injection of 7.8 pmoles of BOM. Higher doses of i.v. BOM did produce phasic and tonic GP changes. Doses of 7.8 pmoles in the NTS increased tonic GP by 0.5 ± 0.1 cm $H_2O$ and produced a maximal increase in the number of giant phasic waves greater than three times the mean phasic wave amplitude, from 0.7 ± 0.2 to 10.3 ± 2.8 per 5 minute period (n=4). A 7.8 pmoles dose of i.v. BOM did not change the number of phasic giant waves but increased tonic GP by 1.0 ± 0.2 cm $H_2O$ (n=4). Spinal cord transection and bilateral cervical vagotomy blocked tonic and phasic gastric changes following any dose of BOM injected into the NTS; however, GP responses after doses of 7.8 pmoles of i.v. BOM were not blocked. These data suggest that BOM may act both centrally (NTS) and peripherally to modulate tonic GP. BOM may modulate phasic gastric activity primarily through mechanisms in the NTS.

SES is the recipient of a post-doctoral fellowship through NIH HL07121. WTT is an Established Investigator for the American Heart Association.

222.14 PREGANGLIONIC PARASYMPATHETIC NEURON PROJECTIONS FROM THE DORSAL MOTOR NUCLEUS OF THE VAGUS TO THE STOMACH AND SMALL INTESTINE IN CAT AND RABBIT. Y. Torigoe*, D.G. Lontok*, F.A. Magarro*, R.D. Cernucan*, J.S. Nishimoto* and R.H.I. Blanks. Depts. of Anatomy and Surgery, Coll. of Med., Univ. of Calif. Irvine, Irvine, CA 92717.

Motion sickness, a multisymptom disorder characterized by abnormal gastrointestinal motility and emesis is, in part, induced by vestibular effects upon the parasympathetic outflow of the gut. The location of preganglionic parasympathetic neurons projecting to the abdominal viscera was examined using the technique of retrograde transport of horseradish peroxidase (HRP). HRP was injected into, or exposed to: 1) the sub-diaphragmatic part of the anterior vagus nerve; 2) the stomach, and 3) portions of the small intestine (duodenum, jejunum, and ileum). The vast majority (97%) of the preganglionic parasympathetic neurons from the vagus nerve are located within the dorsal motor nucleus of the vagus (DMV). The remainder are found in the nucleus of the tractus solitarius, the nucleus ambiguous and the nucleus retroambigualis. The components of the anterior vagus at the level of the diaphragm arise equally from the left and right nuclei as do the projections to the somach and small intestine. Within any one section, the labelled neurons of the DMV tend to cluster within the medial, central or lateral part of the nucleus, but consideration of the total labelling shows no indication of a systematic medial-to-lateral topography. However, there are strong rostral-to-caudal labelling differences which depend upon the location of the injection site. More proximal organs (e.g., stomach, duodenum) receive a greater number of fibers from the rostral portion of the DMV, whereas more distal organs (e.g., jejunum, ileum) receive fewer efferents and these arise predominantly from the caudal portion of the DMV. These data suggest that the DMV is viscerotopically organized, finding which should be considered in future studies of the brainstem control of visceral reflexes and parasympathetic outflow during vestibularly induced motion sickness. (Supported by NASA Grant #NAG2-288 to RHIB. YT is a recipient of NASA Research Associate Award #MAGW-70.)

222.15 IONTOPHORETIC CHARACTERIZATION OF THE HYPOTHALAMIC AND MESENCEPHALIC NEURONS RESPONDING TO GASTRIC DISTENSION, AFTER THE VAGAL TRANSECTION. I. Zarco de Coronado, F. C. Barone and M. J. Wayner. Depto. de Fisiología, Fac. de Medicina, 70250, UNAM, México and Brain. Res. Lab. 601 University Ave. Syracuse, N. Y. 13210.

We have previously reported that mesencephalic and hypothalamic neuronal responses were transiently attenuated after the transection of the bilateral cervical vagus. The observations here reported were planed to obtain evidence from unit recordings for chemosensitive changes as underlaying mechanisms of these vagal effects.

In urethane anesthetized rats gastric distension was produced by introducing 5-10 ml of water at 20-38ºC, during 20-30 sec, in a rubber balloon placed into the stomach. Extracellular action potentials were recorded through the central barrel of a seven barrel microelectrode. They were amplified by a high input impedance preamplifier and audioamplifier and displayed in conventional oscilloscope and ratemeter. Five of the outer six barrels were respectively filled with 2M monosodium glutamate (Glut) 2M acetylcholine chloride (ACh) 0.5M noradrenaline bitartrate (NA) 1M gamma aminobutyric acid (GABA) 0.5M dopamine HCl (DA) or some specific blockers. The resting outing barrel was filled with 2M sodium chloride and was used for current balancing. Dose response relations for each chemical and gastric distension effects were determined before and after the bilateral cervical vagus transection. Ocasionally the chemicals were applied during the gastric distension.

Both, the gastric distension and the topical stimulation reproduced, in the above mentioned areas of the CNS, the already described effects. The transection of the vagus, besides to attenuate the response to the visceral stimulation, produced an increase or decrease, in different proportion, in the responses to the neurotransmitters. ACh was especially affected in that case.

We conclude that these alterations in the effectiveness of the neurotransmitters to induce the mesencephalic and hypothalamic responses as a consequence of the interruption of the afferent vagal information, may determine, in this condition, the modification of the neuronal responses to the gastric distension.

222.16 PARAVENTRICULAR NUCLEUS MICROSTIMULATION, LESION, AND OXYTOCIN EFFECTS ON GASTRIC VAGAL NEURONS R.C.Rogers and G.E.Hermann Department of Physiology, Univ. Nevada Sch. Med., Reno, NV 89557

The hypothalamic paraventricluar nucleus (PVN) is known to make direct, monosynaptic connections with preganglionic parasympathetic neurons in the medulla as well as sympathetic neurons in the lateral horn of the spinal cord. Additionally, the PVN makes substantial direct projections with visceral afferent "relay" nuclei such as the nucleus of the solitary tract (NST). The PVN is, therefore, in position to regulate autonomic function by a) directly controlling autonomic outflow via action on the preganglionic neurons or b) altering the gain or setpoint of autonomic reflexes by changing the response properties of neurons receiving visceral regulatory input. Furthermore, oxytocin appears to be an excellent candidate peptide neurotransmitter for these PVN autonomic pathways.

As part of our continuing studies of PVN regulation of gastric function, we have found that a) gastric acid secretion, in response to vagal sensory stimulation is suppressed following unilateral PVN lesions on the side of the sensory input and b) PVN microstimulation markedly enhances the response of NST neurons to gastric inflation. The responsible pathway is probably monosynaptic. In addition, we have developed a composite glass-metal pico-injection/recording electrode which allows us to inject sub-picomolar amounts of putative peptide neurotransmitter onto small, identified neurons. Using this arrangement, we have collected preliminary evidence that electrophysiologically-identified gastric dorsal vagal neurons are activated by extremely small (850fm) amounts of oxytocin injected in their vicinity.

This evidence, together with previous data collected in other laboratories as well as our own, suggest that the PVN may control gastric or other autonomic functions by controlling both the output of autonomic "motor" neurons as well as the sensitivity of the afferent limb of reflex arcs which "drive" these motor neurons.

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222.17 INCREASED HEXOKINASE ACTIVITY IN THE HYPOTHALAMUS OF RATS WITH DIABETES INSIPIDUS. F.R. Calaresu, W.E. Turton* and T.L. Krukoff. Dept. of Physiology, Univ. of Western Ontario, London, Canada N6A 5C1.

Two commonly used methods to study metabolic activity in discrete areas of the central nervous system under different physiological conditions are the 2-deoxyglucose (2-DG) technique, which provides information about the activity of the first step of the glycolytic pathway, and the cytochrome oxidase (COX) technique which provides an index of general metabolism. Another method that may also be used as a marker of metabolic activity is the measurement of hexokinase (HK) activity, which demonstrates glucose utilization in the brain. Experiments were done to compare HK activity in the hypothalamus of rats with diabetes insipidus (DI) (N=6) and of Long-Evans rats which were water deprived (WD) for 2 days (n=4) with that of control rats (n=4). The animals were anesthetized (sodium pentobarbital, 50 mg/kg i.p.) and perfused transcardially with saline; the brains were removed, frozen, sectioned (18 μm) on a cryostat and mounted on glass slides. Transverse forebrain sections were processed for HK histochemistry as described previously (Acta Histochem., 28: 286, 1967). Changes in HK activity were assessed by counting the number of neurons containing HK activity above background and by estimating the area of increased activity using a Zeiss Videoplan Microprocessor connected to a Zeiss microscope. HK activity was significantly increased bilaterally in the caudal aspect of the supraoptic nucleus (SON) in DI (15.8 ± 1.4 cells in a representative transverse section) and in WD rats (18.0 ± 1.1 cells) compared to control animals (6.8 ± 1.7 cells). No significant change in HK activity of the paraventricular nucleus of the hypothalamus (PVH) was observed. These results suggest that the increased HK activity of the caudal SON in DI and WD rats was associated with hyperactivity of the cells in this region which normally produce vasopressin. The lack of change in HK activity in the PVH compared to the caudal SON could be due to a lower number of vasopressin neurons in the PVH (J. Neur. Transmiss., 36: 195-215, 1975). These results in DI rats are in partial agreement with those obtained using COX histochemistry (Brain Res., 280: 160, 1983), but are at variance with the results obtained using 2-DG (Brain Res., 275: 189, 1983). It has been suggested that the 2-DG technique demonstrates metabolic changes primarily in nerve terminals (Science, 205: 723, 1979) while COX and HK histochemistry appear to show the level of metabolic activity in cell bodies more clearly than the 2-DG method. The present experiments demonstrate that HK histochemistry may be used as a marker of metabolic activity in discrete regions of the central nervous system.

(Supported by the Medical Research Council of Canada).

222.18 THE PARAVENTRICULAR NUCLEUS IS NOT REQUIRED FOR INHIBITION OF GASTRIC ACID SECRETION FOLLOWING INTRACISTERNAL BOMBESIN. M.W. Gunion and Y. Taché. Center for Ulcer Research and Education, Wadsworth Vet. Adm. Med. Ctr., Los Angeles, CA. 90073; and Dept. of Medicine, U.C.L.A., Los Angeles, CA 90024.

Injections of bombesin into either the paraventricular nucleus or the cisterna magna suppress gastric acid secretion. To determine if the paraventricular nucleus has any role in the mediation of the effect of intracisternally administered bombesin, bombesin was administered intracisternally to rats which had previously received electrolytic lesions of the paraventricular nucleus.

Under ether anesthesia, bilateral lesions of the paraventricular nucleus were made in adult male albino rats (232-285 g) using a platinum electrode (400 u diameter) insulated except for the conical tip (500 u exposed) and 1.0 mA anodal current of 20 s duration. On the third day after lesioning, food, but not water, was removed. On the fourth day after lesioning, rats were anesthetized with ether and given intracisternal injections of bombesin-14 (500 ng) or vehicle (0.1% BSA in 0.9% NaCl; both 10 ul). A ligature was immediately placed around the pylorus through a small abdominal incision. Exactly 2.0 hr postinjection the rats were decapitated, and gastric contents, blood, and the brain were saved. Lesion placement was confirmed by assessment of 50 u brain sections stained with thionin.

Paraventricular nucleus lesions did not alter ($p>.05$) the decreases in gastric acid concentration, secretion volume, and total acid output, or the increases in gastric acid pH and serum gastrin, which followed bombesin administration (bombesin effects all $p<.05$). Enhanced acid secretion in lesioned rats ($p<.01$) appeared to be more likely due to damage of areas adjacent to the paraventricular nucleus, rather than to destruction of the nucleus itself.

The results suggest (1) that the paraventricular nucleus is not involved in bombesin-induced inhibition of gastric acid secretion, and (2) that there is more than one central nervous system site at which bombesin may inhibit acid secretion.

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222.19 EFFECTS OF VENTROMEDIAL HYPOTHALAMIC LESIONS ON THE CEPHALIC PHASE OF GASTRIC ACID SECRETION. William L. Parkinson* and Harvey P. Weingarten. Department of Psychology and Intestinal Disease Research Unit, McMaster University, Hamilton, Ontario, Canada.

The ventromedial hypothalamus (VMH) is implicated as a brain site exerting an inhibitory influence on gastric acid secretion. For example, VMH lesions produce elevations in basal gastric acid output (Weingarten et al., Am. J. Physiol. 239:G221, 1980). Although the effects of VMH lesions on basal acid secretion are well characterised, little is known about VMH control of the cephalic phase of acid secretion. The available data are inconsistent: VMH lesions have been reported to exaggerate (Weingarten et al., Am. J. Physiol. 239:G221, 1980) or eliminate (Ridley et al., Am. J. Physiol. 209:319, 1965) the cephalic phase of gastric acid secretion. Experiments were conducted to further analyze the effects of VMH lesions on this response.

Rats were implanted with chronically-indwelling gastric cannulae to permit measurement of acid secretion in the unanesthetized state. Some animals sustained bilateral electrolytic lesions of the VMH; others served as sham-operated controls.

In the first experiment, the cephalic phase of gastric acid secretion was elicited by peripheral injection of 2-deoxy-glucose (2DG) or insulin. The absolute acid response of VMH rats to 2DG (100 mg/kg) was larger than controls. This difference was eliminated when the stimulated response was corrected for the increased basal secretion characteristic of VMH animals. Insulin produced a dose-related increase in acid secretion in controls. In contrast, VMH rats produced responses that were similar to controls at the lowest dose of insulin (.25 U/kg) but failed to show increases in acid secretion to higher doses (.50 & .75 U/kg).

In a second experiment, the cephalic phase of acid secretion was activated by the anticipation of eating. Food-deprived rats were trained to expect a meal in a distinctive environment. Prelesion, all animals showed large gastric acid responses when placed in this environment. VMH lesions significantly reduced the magnitude of the anticipatory response even though behavioural data indicated that these animals continued to expect the food.

These data demonstrate that VMH lesions have different effects on the cephalic phase of gastric acid secretion depending on the procedure used to activate that response.

222.20 GASTRIC DISTENSION MODIFIES THE ELECTRICAL ACTIVITY OF THE OLFACTORY BULB. D. E. García-Díaz*, H. U. Aguilar-Baturoni, R. Guevara-Aguilar and M. J. Wayner. (SPON: L. Cintra McClone). Depto. de Fisiología, Facultad de Medicina, UNAM, México 04510, D. F., and Division of Life Sciences, University of Texas at San Antonio, San Antonio, TX. 78285.

In a previous paper we reported that olfactory bulb cells responded to vagal nerve stimulation (García-Díaz, D. E., Brain Res. Bull., 12 (5): 529-537, 1984). The aim of this work was to demonstrate that influences from the stomach modifies the electrical activity of the olfactory bulb.

Unitary discharges in the olfactory bulb were extracellularly recorded in 10 Wistar and 7 Sprague-Dawley rats, anesthetized with chloral hydrate 400 mg/Kg or urethane 1.5 g/Kg body weight, while gastric distension was performed by means of a pediatric urinary latex catheter inserted through the esophagus into the stomach: 37 neurons located in the periglomerular layer of the olfactory bulb, were sensitive to gastric distension; 12/37 units responded with inhibition alone, 8/37 exhibited inhibition-excitation effect and 17/37 responded with tonic or phasic activation. Generally, the latency of responses to gastric distension was inferior to one second. All those cells which responded to gastric distension, responded also to vagus nerve stimulation; this effect was suppressed by the section of the vagus nerve at the cervical level. Three ml was the threshold volume infused in the gastric camera that was necessary to get an inhibition discharge activity of the olfactory bulb neurons, but when more than 8 ml were infused, increase in their firing rate was observed.

The results of the present experiment indicate that the discharge frequency of the olfactory bulb neurons, and particularly the periglomerular cells, are modulated by visceral afferents.

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222.21 EFFECTS OF THE INTRATHECAL ADMINISTRATION OF SUBSTANCE P ON BLOOD PRESSURE, HEART RATE AND ADRENAL SECRETION OF EPINEPHRINE. C.L.Riphagen, L.Bauce*, W.L:Veale and Q.J. Pittman. University of Calgary, Calgary, Alberta, Canada T2N 4N1

Stimulation of the ventral medulla causes the release of substance P (SP) at spinal levels, possibly from descending SP fibres which have their cell bodies in the brain stem. This release is accompanied by increases in blood pressure (BP) and heart rate (HR) which can be abolished by intrathecal (IT) administration of a SP antagonist. SP immunoreactive fibres surround the adrenal preganglionic neurons (PGN) in the intermediolateral nucleus (IML) of the spinal cord. Large doses of SP, given IT, have previously been reported to increase the levels of circulating catecholamines. In this study we have attempted to correlate the release of epinephrine (EPI) from the adrenal glands, in response to IT SP, with the accompanying increases in BP and HR.

Male Sprague-Dawley rats (250-350g) were anesthetized with Inactin(0.12g/kg i.p). An IT cannula was passed through an incision in the atlanto-occipital membrane and threaded down the subarachnoid space to the T9-T11 region. The trachea was intubated and a cannula (PE50) was inserted into the left carotid artery to monitor BP and HR. The adrenal vein was exposed via a midline incision. After a 30 min. equilibration period, two 30ul blood samples, taken 10 min. apart, were obtained directly from the adrenal vein using an heparinized microcapillary tube with the tip pulled to a sharp point. These samples were used to obtain baseline EPI values. IT injections of SP(7.4nmoles, n=5; 100pmoles, n=5) or vehicle only (5-ion solution, n=5) were made in 15 animals. Blood samples were obtained 2, 15 and 30 minutes after IT injections. All blood samples were centrifuged immediately and the plasma portion was stored at -20°C prior to assay for EPI, using HPLC/EC detection.

Results were as follows:

| | max Δ BP (systolic) mmHg | max Δ HR b.p.m. | % Δ EPI after 2 min. |
|---|---|---|---|
| CONTROL | + 0.2 ± 0.66 | 0 | + 9.75 ± 18 |
| SP,100pmoles | + 18.4 ± 5 | + 23 ± 6.6 | + 98.4 ± 32 |
| SP,7.4nmoles | + 34.7 ± 7 | + 35 ± 9.4 | + 509 ± 112 |

Values = mean± s.e.m.

These results suggest that the cardiovascular responses to spinal SP may be mediated in part by the increased output of adrenal EPI as a result of activation of adrenal PGNs in the IML.

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222.22 RELATIONSHIP BETWEEN NORADRENERGIC AND SEROTONERGIC UNIT ACTIVITY AND THE CARDIAC CYCLE IN AWAKE, FREELY MOVING CATS. D.A. Morilak, C. Fornal, S. Auerbach, and B.L. Jacobs, Prog. in Neurosci., Dept. of Psych., Princeton Univ., Princeton, NJ 08544.

Noradrenergic (NE) neurons of the locus coeruleus (LC) and serotonergic (5HT) neurons of the raphe nuclei dorsalis (NRD) and magnus (NRM) have been hypothesized to play a role in cardiovascular regulation. We have thus examined the discharge of these neurons in relation to the cardiac cycle in awake, freely moving cats. Single-unit recordings of NE and 5HT neurons were obtained by means of chronically implanted flexible microwires. Units were identified on-line by previously described pharmacological and electrophysiological criteria. Unit spikes were digitized with a Schmitt trigger and stored on magnetic tape. The EKG signal was fed through a Schmitt trigger set to deliver a pulse at the peak of the R-wave. Computer-generated peristimulus time histograms of unit activity were then constructed around the R-wave. The activity of 5HT neurons in both NRD and NRM showed no consistent relationship to the cardiac cycle. In contrast, LC-NE neurons showed a consistent pulse-synchronous modulation such that the level of unit activity was least during the 40 ms period preceding the R-wave (during atrial systole), and was maximal from 100-140 ms following the R-wave. The activity of non-NE neurons in LC showed no consistent relationship to the cardiac cycle, excluding mechanical artifact as a possible source of the relationship. These data demonstrate that the excitability of LC-NE neurons is influenced by input from cardiovascular afferents whose activity is phase-locked to the heart beat. Atrial stretch receptors may be involved since they discharge during the observed period of neuronal suppression and are silent when LC-NE activity is highest. Since these receptors regulate blood volume by inhibiting vasopressin release, our results are consistent with previous observations that increases in blood volume suppress LC-NE unit activity in the anesthetized rat, and that the activity of these neurons may facilitate vasopressin release. Our results indicate that the activity of LC-NE neurons in unanesthetized, behaving animals can be influenced by physiological variables operating within their normal ranges. This supports the hypothesis that LC-NE neurons may play a role in the regulation of the cardiovascular system, perhaps as part of a more general CNS modulatory function. Supported by NIMH grant MH 23433 and a NSF pre-doctoral fellowship to D.A.M.

222.23 RESPONSE OF SEROTONERGIC DORSAL RAPHE NEURONS TO ENVIRONMENTAL HEATING AND PYROGEN ADMINISTRATION IN FREELY MOVING CATS. C. Fornal, D. Morilak, S. Auerbach and B.L. Jacobs. Prog. in Neurosci., Dept. of Psychol., Princeton Univ., Princeton, NJ 08544.

Serotonergic neurons of the dorsal raphe nucleus (DRN) have been hypothesized to play a role in thermoregulation. These neurons project to the anterior hypothalamic-preoptic area where microinjection of serotonin evokes thermoregulatory responses. Several studies have examined the relationship between body temperature and dorsal raphe unit activity in anesthetized animals. In general, previous results have been equivocal or contradictory. Since anesthesia affects both unit responses and central thermoregulation, we examined the effects of acute environmental heating and pyrogen-induced fever on serotonergic DRN cells in unanesthetized freely moving cats. Single unit activity was recorded using movable electrode bundles consisting of 32 and 64 μm dia. insulated, nichrome wires implanted in the DRN. Serotonergic neurons were initially identified by their slow and regular discharge pattern and complete cessation of activity both during REM sleep and in response to the specific serotonin agonist 5-methoxy-N,N-dimethyltryptamine (250 μg/kg, i.m.). After obtaining baseline unit activity, the temperature in the recording chamber was raised to 43°C and maintained until cats displayed continuous panting for one min (about 30-40 min after the onset of heating). Serotonergic unit activity was not significantly changed from baseline at any time during the period of environmental heating. We also examined the unit activity of serotonergic DRN neurons in response to the synthetic pyrogen muramyl dipeptide (MDP; 50 μg/Kg, i.v.). MDP produced an increase in body temperature within 30 min. The peak elevation ranged from 2.0 to 2.5°C above baseline and occurred 1-2 h post-injection. Body temperature returned to baseline levels within 6 h. Cats appeared lethargic and slept most of the time during the peak febrile response. Serotonergic DRN units showed no significant change in activity following MDP administration. These results indicate that the thermoregulatory responses associated with both environmental heating and pyrogen-induced fever are not accompanied by changes in serotonergic DRN unit discharge. This, however, does not exclude a role for these neurons in thermoregulation since serotonin release may be modulated directly at the axon terminal. Supported by NIMH grant MH 23433 and postdoctoral fellowship MH 08869.

222.24 RELATION OF BODY TEMPERATURE TO AMBIENT TEMPERATURE OF CATS WITH DORSOLATERAL PONTINE TEGMENTAL LESIONS. L. Amini-Sereshki and A. R. Morrison. Dept. of Animal Biology, Sch. of Veterinary Medicine, University of PA, Philadelphia, PA 19104 U.S.A.

Small bilateral dorsolateral pontine tegmental lesions induce paradoxical sleep (PS) without atonia (Henley, K. and A.R. Morrison, Acta Neurobiol. Exper. 34:215-232, 1974). Cats in PS without atonia do not exhibit heat-gain responses, such as shivering, piloerection, or assumption of a protective curled body when placed in the cold (Hendricks, J.C., Exp. Neurol., 75:700-710, 1982), demonstrating in a vivid way the suppression of thermoregulation that characterizes PS (Parmeggiani, P.L., Physiol. in Sleep. J. Orem ed., Acad. Press, New York, 97-143, 1980). These cats also show an alteration of thermoregulatory responses to thermal load during wakefulness. When placed at cold ambient temperatures ($T_a$), they start to shiver, piloerect, and curl their bodies at 15-17°C. Intact cats start to shiver at a $T_a$ of 8-10°C. At high $T_a$, the lesioned cats begin panting at a lower $T_a$ (33-35°C) than intact cats do, suggesting that the thermoneutral zone narrowed (Amini-Sereshki, L. and A.R. Morrison, Soc. for Neurosci. Abstr., 9:112, 1983).

The present experiments initiate a search for reasons for the increased sensitivity to ambient temperature of these lesioned cats. We recorded the brain temperature ($T_b$), measured in the depths of the cerebral cortex via thermistors, as an index of body temperature, at different $T_a$s during wakefulness. $T_b$s were recorded on several trials at $T_a$s between 5 and 40°C before (N=1) and after (N=2) placement of pontine lesions. Each session consisted of 30 minutes exposure to a fixed $T_a$.

The $T_b$s of cats with lesions, like those of normals, did not drop when the cats were exposed to low $T_a$s for 30 minutes. At room temperature the $T_b$ of a lesioned cat was also similar to that of the intact animal. $T_b$ rose in both intact and lesioned cats exposed to high $T_a$s. The rise of $T_b$, however, started at a lower $T_a$ in the lesioned cats, and it rose to a higher value than that of the intact cat at the same $T_a$.

Results of the experiments at low $T_a$s suggest that the lesions resulted in a greater influence of skin temperature on hypothalamic sensitivity at an as-yet-undetermined level of the neuraxis or the periphery. Since the body temperature did not remain at the same level as that of the intact animal at high $T_a$s, lesions appear to have altered hypothalamic control over lower level thermoregulatory mechanisms, either by damaging pathways or cells locally involved in thermoregulatory mechanisms.

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222.25 EFFECTS OF COLD EXPOSURE ON BRAIN LEVELS OF VASOPRESSIN AND OXYTOCIN IN THE RAT. N.W. Kasting. Dept. Physiol. Fac. Med., Univ. British Columbia, Vancouver, B.C. V6T 1W5

Experimental evidence has suggested that vasopressin (AVP) may be a neurotransmitter in thermoregulatory pathways subserving fever production. Its specific role may be to inhibit heat gain mechanisms in the septal (SEP) or diagonal band (DB) areas of the brain, areas in which there are AVP-containing nerve terminals and AVP receptors. No specific function for oxytocin (OXY) has been proposed in thermoregulatory pathways but it is also found in these areas of the brain. We sought to determine if levels of AVP or OXY changed in certain brain areas upon cold exposure. AVP-containing cell bodies are located in supraoptic nucleus (SON), paraventricular nucleus (PVN), suprachismatic nucleus (SCN) and bed nucleus of the stria terminalis (BST). Nerve terminals of AVP are found in DB, SEP, and PIT. The preoptic/anterior hypothalamus (POA) is the area most intimately involved in integration and regulation of thermoregulatory responses.

Wistar male rats were assigned randomly to control or cold exposed groups. The cold exposed group were kept for 3 days at 6°C. The animals were then quillotined, brains frozen and discrete areas removed for extraction and assay of AVP and OXY.

AVP levels increased relative to controls in DB, SEP, and SCN. OXY levels increased only in DB. No changes were observed in plasma, PIT, POA, SON, PVN or BST. Control animals showed a significant positive correlation in AVP content between certain areas of the brain: SON vs PIT; SON vs SCN; BST and POA; and DB vs SEP. These correlations disappeared in cold exposed rats but were replaced with positive correlations between: SON vs POA; and PVN vs POA.

The increases in AVP content in SEP and DB areas (suggesting decreased release) where AVP has been demonstrated to inhibit heat gain effectors is of considerable interest since cold exposure causes maximal functioning of heat gain effectors. The meaning of the observed changes in correlation of AVP content between brain areas is not yet clear although involvement of the POA, an important thermoregulatory center in the brain, in these changes is of compelling interest.

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222.26 THE ROLE OF β-ADRENERGIC RECEPTORS IN ADRENAL CORTICAL CELL PROLIFERATION. M.A. Holzwarth, B. Bartnicke*, N. Kleitman, Department of Anatomical Sciences and Program in Neural and Behavioral Biology, University of Illinois, Urbana, IL 61801

The autonomic nervous system mediates the compensatory adrenal cortical growth which occurs in the remaining adrenal following unilateral adrenalectomy. This growth, a proliferative response of cells of the zona glomerulosa, is measurable by increased adrenal weight and DNA, RNA and protein content. Evidence for the role of the sympathetic nervous system in the mediation of this response has been recently reported. The adrenal cortex is innervated by catecholaminergic nerves which are eliminated by neonatal chemical sympathectomy. Sympathectomy prevents the compensatory adrenal cortical growth response to unilateral adrenalectomy (Am. J. Physiol. 248:E261). The mechanisms and adrenoreceptors by which the sympathetic nervous system affect this response remain unelucidated.

In vitro incorporation of $^{3}$H-thymidine into adrenal DNA was found to be a more sensitive and reliable measure of cell proliferation than the previously utilized measure of total DNA content. A time course of compensatory growth was established: A significant increase in the rate of DNA synthesis could be demonstrated 18, 24, 48 and 72 hrs after unilateral adrenalectomy, but not after 6, 12 or 120 hrs. To investigate the role of adrenoreceptors in this response, β-adrenergic agonist, isoproternol (5 mg/kg BW) was administered for 24 hrs. While isoproterenol has been reported to stimulate cell proliferation in other tissues, in the adrenal it significantly decreased (44%) $^{3}$H-thymidine incorporation into DNA relative to the saline-injected controls ($p<0.01$). Furthermore, isoproterenol administration inhibited the usual increase in the rate of DNA synthesis observed 24 hrs after unilateral adrenalectomy. These results are corroborated by the observation that blockade of β-adrenoreceptors with propanolol (10 and 25 mg/kg) or Sotolol (25 and 50 mg/kg) resulted in increased compensatory adrenal cortical growth relative to saline-injected controls ($p<0.05$). Thus, β-adrenergic receptors appear to inhibit DNA synthesis. We conclude from these results that activation of β-adrenergic receptors is not the mechanism by which catecholaminergic neurons stimulate cell proliferation in the compensatory growth response. But rather, it is possible that the trigger for compensatory adrenal growth involves a release from this β-adrenergic inhibition. (Supported by NSF PCM-8109756 and NIH PHS-5T32GM07).

222.27 PLASMA ACTH AND ADRENAL CATECHOLAMINE SECRETORY RESPONSES TO TRIGEMINAL SUBNUCLEUS CAUDALIS STIMULATION: EFFECT OF STIMULUS PATTERN. D.A. Bereiter, W.C. Engeland* and D.S.Gann. Brown U., R.I. Hospital, Endocrine Lab, Providence, R.I. 02902

Trigeminal subnucleus caudalis(TC) is a primary relay nucleus for nociceptive afferent input from the head and facial regions. Neurons within the magnocellular (deep) portions of TC project rostrally to the diencephalon and caudally to the spinal cord. The goal of this study was to assess the effect of the pattern of electrical stimulation(ES) of TC on the release of ACTH and on adrenal catecholamine secretion, hormonal responses which are presumed to accompany nociceptor neuron activation.

Adult cats were anesthetized with chloralose/urethane after induction with ketamine. Catheters were placed in the descending aorta(arterial blood pressure), femoral vein(ACTH concentration), brachial vein(drug and dextran infusions), and the lumboadrenal vein(adrenal catecholamine secretion). After tracheostomy, animals were paralyzed with gallamine and artificially ventilated. Animals were placed in a stereotoxic frame using blunt ear bars and the dorsal surface of the caudal medulla was exposed for electrode (concentric bipolar) placement using the obex as the reference point. Cathodal stimulation consisted of 300 pulses delivered over 15sec (75μA, 0.2ms duration, 20cps) in a continuous pattern (50ms intervals) or in a burst pattern(10ms intervals). All cats received both stimulus patterns at each electrode site. Blood samples were collected at -5,0,1,3 and 6min and assayed for ACTH by RIA and adrenal catecholamines by HPLC-EC.

The results indicate that stimulation of the magnocellular TC evoked an increase in ACTH(>30pg/ml) by +1min regardless of stimulus pattern. ACTH returned to baseline by +6min after continuous pattern ES, but remained elevated at +6min after the burst pattern ES. The mean prestimulus adrenal epinephrine secretion($\bar{E}$) and norepinephrine secretion($\bar{NE}$) rates were 16.5±4.7 and 29.4±5.5ng/min, respectively.TC-ES by continuous pattern did not affect adrenal $\bar{E}$, whereas stimulation by burst pattern caused a significant increase in adrenal $\bar{E}$(+26.8±12ng/min). Continuous pattern TC-ES caused a variable change in adrenal $\bar{NE}$, whereas the burst pattern evoked a consistent increase in adrenal $\bar{NE}$(+17±4.3ng/min). The mean ratio of $\bar{E}/\bar{NE}$ was 0.64 ± 0.21 prior to TC-ES and was not altered by continuous pattern TC-ES, whereas the burst pattern TC-ES evoked an increase (by +1min) in the $\bar{E}/\bar{NE}$ ratio. Arterial pressure decreased slightly (-4.7±3.3mmHg) after continuous pattern TC-ES, whereas burst pattern caused a slight increase (+4.9± 3.7mmHg).

We conclude that the magnocellular laminae of TC contains neural elements which contribute to the control both of ACTH and of adrenal catecholamine secretion. These data indicate that stimulation of the magnocellular TC evokes a prompt increase in plasma ACTH independent of stimulus pattern. In contrast, adrenal $\bar{E}$ is sensitive to the pattern of stimulation since burst pattern stimuli caused a greater increase in adrenal $\bar{E}$ than was seen after continuous pattern stimuli. This study was supported by NIH Grants AM-26831 and GM-27946.

222.28 DIFFERENTIAL SYMPATHOADRENAL CATECHOLAMINE RESPONSES TO CENTRAL AND PERIPHERAL NICOTINE. G.R. Van Loon, J.A. Kiritsy-Roy* and S.A. Mousa*. VA Medical Center and Departments of Medicine and Pharmacology, University of Kentucky, Lexington, KY 40511.

Nicotine increases catecholamine secretion through its peripheral effects on sympathetic ganglia and nerves and on adrenal chromaffin cells, but brain-mediated effects of nicotine on catecholamine secretion have not been described. In this study, we compared the effects of centrally- and peripherally-administered nicotine on plasma concentrations of catecholamines. Furthermore, since tolerance to the effects of nicotine has been described, we also compared the effects of initial and subsequent central and peripheral injections of nicotine on catecholamine secretion. Adult male Sprague-Dawley rats were prepared at least two days before experimentation with an indwelling carotid cannula housed in a flexible spring which allowed drug administration and blood sampling to be carried out without the stress of handling. In some rats, a stainless steel guide cannula was stereotaxically positioned 1.5 mm above the right lateral brain ventricle at the same time. For studies of peripheral nicotine, either nicotine 0.5 mg/kg or saline was administered intraarterially three times at 70 min intervals. For studies of central nicotine, animals were handled briefly to lower a drug-filled injector through the guide cannula into the lateral ventricle. Thirty min later, 2 μl of test solution was microinjected over 2 min. Nicotine 30 nmol was administered intraventricularly, and a time course was established. Then, nicotine 30, 60, or 120 nmol was injected daily for 7 days, and blood was sampled before and at the time of peak response, 2 min after nicotine. The initial peripheral administration of nicotine increased plasma concentrations of both norepinephrine (NE) and epinephrine (EPI) markedly; NE increased by 30.0 ± 4.6 nM and EPI increased 14.7 ± 3.0 nM. These catecholamine responses were blocked by the simultaneous administration of hexamethonium. The initial central administration of nicotine produced dose-related increases in plasma EPI, but in contrast to peripheral nicotine only the highest central dose tested increased plasma NE concentration. Nicotine 120 nmol intraventricularly increased plasma NE by 1.7 ± 0.6 nM and plasma EPI by 13.0 ± 3.2 nM. That is, this central dose of nicotine produced an equal EPI response but only about 1/20 of the NE response produced by peripheral nicotine. Thus, central administration of nicotine appeared to stimulate predominantly sympathetic outflow to the adrenal medulla with very little or no effect on outflow to sympathetic nerves. In contrast, peripheral administration of nicotine appeared to be associated with stimulation of both adrenal medulla and sympathetic nerves. Furthermore, tolerance was demonstrated for the NE responses to both central and peripheral repeated nicotine administration, but tolerance for the EPI response was demonstrated only to central administration of nicotine.

222.29 COMPENSATORY ADRENAL CORTICAL GROWTH OCCURS AFTER SUPPRESSION OF ANGIOTENSIN II. N. Kleitman and M. A. Holzwarth. Neural and Behavioral Biology Program and Department of Anatomical Sciences, University of Illinois, Urbana IL 61801

Recently, we reported that the zona glomerulosa of the rat adrenal cortex is innervated by catecholaminergic fibers. Elimination of these nerves by chemical sympathectomy inhibited compensatory adrenal growth, a neurally mediated, proliferative response to unilateral adrenalectomy. Because angiotensin II (AII) stimulates adrenal cortical cell proliferation in vitro, and sympathectomy may affect the renin-angiotensin system, we tested whether suppression of AII prevents the compensatory adrenal growth response.

The angiotensin converting enzyme inhibitor, Enalapril (20 mg/kg per day) was administered to rats in their drinking water for 4 or 7 days. Control animals drank tap water. The efficacy of this treatment was demonstrated by the reduced blood pressure response of treated rats to exogenous angiotensin I relative to their response to AII. Rats were unilaterally- or sham-adrenalectomized on the second or fourth day of Enalapril treatment and were sacrificed 72 hrs later. Significant increases in adrenal weight, and DNA and protein content of unilaterally adrenalectomized rats in all groups indicated that compensatory adrenal growth was not inhibited by Enalapril treatment. The elevation in adrenal DNA content after adrenalectomy confirmed that this was a cell proliferative response. Thus, suppression of AII synthesis did not inhibit compensatory adrenal growth.

We next tested the effect of elevated potassium intake on compensatory adrenal growth. Potassium directly stimulates adrenal aldosterone synthesis while inhibiting the renin-angiotensin axis. 2.5% KCl solution was substituted for drinking water for 7 days (controls drank tap water). High potassium intake increased plasma aldosterone but not corticosterone levels in treated animals. Rats underwent unilateral- or sham-adrenalectomy on the fourth treatment day. 72 hrs later, adrenal DNA content was significantly increased in potassium treated, adrenalectomized animals, again confirming that cell proliferation had occurred in response to unilateral adrenalectomy, however, these rats showed no significant increase in adrenal weight. We conclude that compensatory adrenal growth was not inhibited in the potassium-treated group, even though adrenal weight measures did not indicate the response.

Our findings that neither AII-suppressing treatment inhibited the compensatory adrenal growth response suggest that this response is not dependent on the mitogenic effects of angiotensin. (Supported by NSF Grant PCM-8109756 and NIH PHS-5T32GM07.)

## RECEPTOR MODULATION: UP AND DOWN REGULATION I

223.1 ASCORBIC ACID TREATMENT INCREASES ACETYLCHOLINE RECEPTOR mRNA LEVELS IN CULTURED MYOGENIC CELLS. D. Knaack*, S. Admon*, T. Podleski*, R. Oswald and M. M. Salpeter. Section of Neurobiology & Behavior, Cornell University, Ithaca, NY 14853.

The addition of crude brain extracts to cells from myogenic cell lines can cause a 2 to 5 fold increase in surface acetylcholine receptors (AChR) (Podleski et al., Proc. Natl. Acad. Sci., 75:2035, 1978). It has been shown that ascorbic acid is the principal component of the brain extract responsible for this effect (Knaack & Podleski, Proc. Natl. Acad. Sci., 82:575, 1985), and commercially available ascorbic acid induces an increase in surface AChR similar to that induced by the brain extracts (Knaack, Shen, Salpeter & Podleski, in press). This increase in AChR levels is not paralleled by an increase in creatine kinase nor is it caused by increased growth, survival, fusion, or total protein synthesis. The increased surface AChR is also not due to a decrease in the AChR degradation rate (Knaack, Shen, Salpeter & Podleski, in press).

As an initial approach to understanding the mechanism of AChR induction by ascorbic acid, we examined AChR mRNA in ascorbic acid-treated cells. Poly(A)$^+$ RNA was prepared from the myogenic cell line (L5), with and without ascorbic acid pretreatment. AChR mRNA was studied on northern blots using a cDNA probe for the mouse α-subunit of the AChR (pA59) (Merlie et al., Proc. Natl. Acad. Sci., 80:3845, 1983). A cDNA probe for rat myosin light chain 2 (p103) (Katcoff et al., Proc. Natl. Acad. Sci., 77:960, 1980) was used as control.

Ascorbic acid-treated cultures had dramatically elevated levels of mRNA for the α-subunit of AChR, while levels of mRNA for myosin light chain 2 were not increased by ascorbic acid treatment.

The ascorbic acid effect is thus most likely due to increased synthesis of AChR resulting from elevated levels of mRNA for the receptor.

223.2 REGULATION OF THE ALPHA(+) AND ALPHA SUBUNITS OF RETINAL NA,K-ATPASE BY TETRODOTOXIN AND RD MUTATION, S. Corey Specht, J. Doval Graziani*, A. Colon Magé*, S. Castro Hernandez* and M. Morales*. Dept. of Pharmacology and Laboratory of Neurobiology, Univ. of Puerto Rico Sch. of Med., San Juan, PR 00936.

The synthesis, degradation and distribution of the alpha subunit of Na,K-ATPase in muscle and kidney is regulated by signals arising from Na-transport work load. Neuronal enzyme has two forms of the catalytic subunit, alpha and alpha(+). To determine if both forms of the neuronal enzyme are subject to down-regulation by decreased Na-transport work load, we examined mice lacking photoreceptor cells (rd) as well as rats and mice pre-treated with intraocular Tetrodotoxin to block action potentials. In rd retinae the number of $^3$H-ouabain binding sites per g tissue was not decreased, although both total retinal weight and binding per retina were only one-third the value in wild type mice. Labeling of alpha and alpha(+) was determined by scintillation counting of SDS-polyacrylamide gels. The incorporation of $^{35}$S-methionine into alpha (+) and alpha isolated from rd retinae was similarly proportional to tissue weight, i.e., one-third control values. On the other hand, intraocular injection of Tetrodotoxin decreased incorporation and prolongedthe half life of alpha(+) relative to alpha. Hence complete absence of action potentials may be required for down-regulation. The differential effect on alpha and alpha(+) suggests the possibility that only one of these two forms, probably alpha, is subject to down-regulation. (Supported in part by NS 07464 and MBS-RR-08102.)

223.3 SEX DIFFERENCE IN $\alpha_1$-NORADRENERGIC RECEPTOR BINDING FOLLOWING ESTRADIOL TREATMENT IN THE GUINEA PIG ASSESSED BY QUANTITATIVE AUTORADIOGRAPHY. A.E. Johnson, B. Nock, B. S. McEwen, and H.H. Feder. Institute of Animal Behavior, Rutgers University, Newark, N.J. 07102 and The Rockefeller University, New York, N.Y. 10021.

Noradrenergic (NA) transmission plays an important role in the regulation of ovarian steroid-dependent LH release and in the regulation of lordosis in female rodents. One way in which ovarian hormones might affect NA function is by altering the number of NA receptors. In this regard, estradiol has been shown to affect one $\alpha$-noradrenergic receptor subtype, $\alpha_2$-receptors, in several brain regions of female guinea pigs. In the present study, we examined the effect of E on a second subtype of NA receptor, $\alpha_1$-receptors. For these studies, we used male and female guinea pigs to evaluate possible sex differences in $\alpha_1$-receptor response to estradiol.

Adult guinea pigs were gonadectomized and treated one week later with either 10µg estradiol-17β benzoate (E) or oil for two consecutive days. Two days after the second injection, animals were decapitated, brains were rapidly removed, frozen on dry ice, and stored at -60°C. Brain sections (30µm) were cut and thaw-mounted onto subbed slides. $\alpha_1$-Receptors were labeled using 1.0nM ($^3$H)prazosin (SA=80.9 Ci/mmole) + 10µM phenylephrine. Tissues were exposed to tritium-sensitive LKB Ultrofilm for 6 weeks. Autoradiograms were analyzed using a computer assisted densitometer that converted optical density to fmoles/mg protein using a standard curve derived from brain mash standards that were coexposed with the labeled brain slices.

In the ventromedial hypothalamic nucleus (VMN) of oil treated animals, we found higher levels of prazosin binding in female guinea pigs than in males. Treatment with E decreased prazosin binding in VMN of females but not males. Prazosin binding in the medial amgdaloid nucleus was found to be higher in females than males, but no effect of E was detected.

These results indicate that E might influence NA transmission in brain by altering the number of $\alpha_1$-receptors in addition to $\alpha_2$-receptors. The mechanism whereby E alters noradrenergic receptors has not yet been determined. Estradiol might alter the synthesis of NA receptors, the level of NA activity, or the coupling of receptors to regulatory proteins. In addition, the possibility of E induced tissue alterations resulting in local changes in autoradiographic efficiency cannot be ruled out at this time. Supported by NS07080 (NIH, B Mc) and HD04467 (NIH, HHF).

223.4 DEPLETION OF EXTRACELLULAR CALCIUM INHIBITS ACETYLCHOLINE RECEPTOR BIOSYNTHESIS. M.A. Wozniak*[1], D.J. Goldman*[2], R.J. Bloch[1], S. Heinemann[2], J. Patrick[2]. [1]Dept. of Physiology, Univ. of Maryland School of Medicine, Baltimore, MD 21201, and [2]Molecular Neurobiology Lab, Salk Institute, San Diego, CA, 92138.

The nonfusing mouse muscle cell line, BC3H1, provides a well controlled experimental system in which to study the regulation of nicotinic acetylcholine receptor (AChR) biosynthesis. When this cell line differentiates, large numbers of nicotinic AChRs are inserted into the surface membrane. In controls (1.8 mM extracellular calcium [$Ca^{2+}$]), appearance of new AChR on the cell surface, as detected by $^{125}$I-alpha bungarotoxin, is linear with time. Incubation of cells in $Ca^{2+}$-depleted medium, or in cycloheximide, results in a complete block in AChR membrane appearance after a 2 hr time lag. Previous experiments have indicated that $Ca^{2+}$ is specifically required in a dose dependent manner for the membrane insertion of AChR. Reduction of $Ca^{2+}$ does not affect AChR degradation and only moderately and non-selectively decreases total cell protein and glycoprotein synthesis. (Soc. Neurosci. Abst., 10:1134, 1984).

When cells depleted of $Ca^{2+}$ are placed in control medium, AChRs reappear on the cell surface at control rates after a 4 hr lag. This reappearance of AChR is completely blocked by the protein synthesis inhibitor, cycloheximide, but only moderately decreased by inhibition of RNA synthesis by actinomycin D. To determine directly if mRNA levels of the AChR subunits are altered during incubation in $Ca^{2+}$-depleted medium, polyadenylated RNA was purified from control, $Ca^{2+}$-deprived and actinomycin-treated cells. The RNA was separated on agarose gels and probed with $^{32}$P-labeled cDNA for the alpha, gamma and delta subunits of the receptor. When control, $Ca^{2+}$-deprived and actinomycin-exposed samples are compared, no difference in steady state mRNA levels of the alpha, gamma or delta subunit is detected.

Transport of assembled AChR to the cells surface does not appear to be altered during $Ca^{2+}$ depletion. First, AChR are transported to the cell surface at control rates during the first two hours of incubation in low $Ca^{2+}$ medium. Second, a 6 hr incubation in $Ca^{2+}$-depleted medium results in a 80% decrease in intracellular $^{125}$I-bungarotoxin binding sites, consistent with a continued transport of AChR from a dwindling intracellular pool.

Incubation in low $Ca^{2+}$ medium blocks AChR biosynthesis at a point which follows mRNA synthesis yet precedes transport of assembled AChR to the cell surface. Reduction of $Ca^{2+}$ may interrupt AChR biosynthesis at (i) translation, (ii) essential cotranslational modification, or (iii) maturation and assembly of the subunits into the mature bungarotoxin binding AChR. Experiments are in progress to distinguish among these alternatives.

223.5 QUANTITATIVE AUTORADIOGRAPHY OF $GABA_A$ RECEPTORS IN RAT FOREBRAIN: RECEPTOR DISTRIBUTION AND EFFECTS OF ESTRADIOL. L. H. O'Connor, B. Nock and B. S. McEwen. The Rockefeller University, New York, NY 10021.

Estradiol has been implicated in the modulation of gamma-aminobutyric acid (GABA) function in brain. One way estradiol appears to regulate GABA activity is through effects on $GABA_A$ receptors (Br. Res. 279, 1983,141: Eur. J. Pharm. 103, 1984, 165), a subtype of GABA receptors that shows a high affinity for GABA agonists in vitro. The present study more precisely localizes the effects of estradiol on $GABA_A$ receptors in rat brain using in vitro quantitative autoradiography. A modification of the method of Palacios et al. (PNAS 77, 1980, 670) was used to label $GABA_A$ receptors in rat forebrain. Following 3 x 10 min. preincubation washes, brain slices were incubated with 20nM $^3$H-muscimol + .1mM GABA. The sections were washed for one min. in ice cold buffer, dried and apposed to LKB tritium sensitive Ultrofilm for 19 days. Areas with the highest concentration of $GABA_A$ receptors included frontal cortex layers 2&3, stratum radiatum, stratum oriens and dentate gyrus. Intermediate concentrations of receptors were found in olfactory tubercle, accumbens, bed nuc. of stria terminalis, anterior hypothalamic area, ventromedial hypothalamic nuc. and compact substantia nigra. Areas with lower concentrations of receptors were found in median preoptic nuc., periventricular hypothalamic nuc., septohypothalamic nuc., arcuate and reticular substantia nigra. This distribution of $GABA_A$ receptors confirms and extends previous observations (Br. Res. 222, 1981, 285).

For investigation of effects of estradiol on $GABA_A$ receptors, ovariectomized female rats were injected with 10ug estradiol benzoate (n=8) or sesame oil (n=7) at 0 and 24 hrs and at 48 hrs after the last injection the rats were decapitated. Using a computer-assisted densitometry system that converts optical density to fmol/mg protein, estradiol was found to affect $GABA_A$ receptors in a number of brain areas. It is noteworthy that some of the areas where receptors were affected are known to concentrate estradiol but effects of estradiol were not limited to those areas. For example, in frontal cortex layer 6, an area that contains few estrogen receptors, estradiol caused a decrease in the concentration of $GABA_A$ receptors. The mechanism by which estradiol altera $GABA_A$ receptors in frontal cortex has not yet been determined. Effects of estradiol on $GABA_A$ receptors in other brain areas is currently under investigation.

223.6 RAPID STEREOTAXIC INJECTIONS INTO MOUSE STRIATUM USING A PLASTIC MOLD. David B. Goodale*, Athole G. McNeil Jacobi*, James D. Winkler* and Benjamin Weiss, Departments of Anesthesia and Pharmacology, Medical College of Pennsylvania, 3300 Henry Avenue, Philadelphia, PA 19129

Microinjections of putative neurotransmitters, neuromodulators and drugs into discrete brain nuclei afford the opportunity of dissecting neurochemical mechanisms in the central nervous system. Most conventional stereotaxic techniques, while highly selective, are tedious and time consuming. We have devised a plastic mold that allows rapid and selective microinjections of compounds into specific brain nuclei of mice. For example, the plastic mold system allows for the unilateral 6-hydroxydopamine-induced denervation of the striatal dopaminergic nerve terminals of 20 mice per hour by one operator. The accuracy of this system resulted in 90% of the mice demonstrating contralateral asymmetry and 70% of the mice having greater than 30 contralateral rotations per five minute test period following the subcutaneous injection of apomorphine (0.5 mg/kg). Analyses were also performed on the drug concentration, rate of injection and type of anesthesia optimum for lesioning dopaminergic nerve terminals with 6-hydroxydopamine. The optimum volume of injectant was one microliter, with larger volumes resulting in the spread of the injectant over the ipsilateral cortex. Concentrations of 6-hydroxydopamine as low as 4 ug/ul were effective in producing contralateral asymmetry. The optimum dose of 6-hydroxydopamine was sixteen micrograms per striatum. The neurochemical specificity of this technique was confirmed by comparing the concentrations of dopamine and serotonin in mouse striatum 2 weeks following the injection of 6-hydroxydopamine. A comparison of different anesthetics showed no significant differences in contralateral rotations following lesioning under Halothane, Chloropent or gamma-butyrolactone anesthesia. This procedure has already been used to study the effects irreversible dopaminergic ligands (Thermos et al., Soc. Neuroscience, 1985), the reversal of 6-hydroxydopamine-induced supersensitivity (Winkler and Weiss, Fourth World Cong. of Biol. Psychiat., 1985) and to differeniate the subtypes of dopaminergic receptors (Goodale et al., Pharmacologist, 1985). In addition, the general technique to be described has obvious applicability for administrating other neurotoxins, radiolabeled tracer substances or even for tissue transplantation. Funded by the Department of Anesthesia, Medical College of Pennsylvania and by the Department of Public Welfare, Commonwealth of Pennsylvania.

223.7 **BETA-ADRENERGIC RECEPTOR AUTORADIOGRAPHY IN RATS TREATED WITH DESMETHYIMIPRAMINE.** E.A.Crisóstomo and J.N.Davis, VA Medical Center and Departments of Medicine (Neurology) and Pharmacology, Duke University Medical Center, Durham, NC 27705.

Chronic treatment with the tricyclic antidepressant desmethylimipramine (DMI) decreases beta(B)-adrenergic receptor binding in brain tissue homogenates, and attenuates the production of cyclic AMP in brain slices exposed to isoproterenol. DMI has long been recognized for its ability to inhibit norepinephrine uptake. In vitro autoradiography is a powerful tool for delineating specific regional changes in neurotransmitter receptor densities. We utilized the B-adrenergic ligand [$^{125}$I]-cyanopindolol (CYP) to define the regional regulation of receptors after DMI treatment.

Adult male Sprague-Dawley rats were given either DMI (15 mg/kg, i.p.) or saline for 12 consecutive days. Twenty-four hours after the last injections, the animals were sacrificed and their brains rapidly frozen. Adjacent serial sections were labeled with 43 pM CYP, and either 50 nM of ICI-118,551 or 70 nM of ICI-89,406 as selective $B_2$- and $B_1$-adrenergic receptor antagonists, respectively. Non-specific binding was estimated after incubation with ±propranolol.

We detected dramatic reductions in quantitative densitometric measurements of $B_1$-adrenergic receptor densities in several regions from brains of DMI-treated rats, including cerebral cortex and hippocampus (pyramidal layer $CA_1$ and subiculum). $B_2$-adrenergic receptors were reduced in the molecular layer of the cerebellum and in layer IV of the cerebral cortex. No appreciable differences in densities were observed in the caudate-putamen, dorsal lateral geniculate nucleus and substantia nigra in both receptor subtypes. In addition, minimal to no change was observed in $B_1$ receptor densities in the cerebellar molecular layer and in $B_2$ receptor densities in layer I of the cerebral cortex and in the hippocampal formation.

These data indicate that 1) both $B_1$- and $B_2$-adrenergic receptor subtypes are affected by DMI treatment, and 2) the decreases in B-adrenergic receptor densities in treated animals are heterogenous within projection areas of locus coeruleus neurons. The heterogeneity of these changes suggests that chronic DMI treatment affects discrete post-synaptic targets of noradrenergic fibers. (Supported by the VA and NS06233)

223.8 ALTERATION OF HYPOTHALAMIC $A_1$ ADENOSINE RECEPTORS IN STRESSED ANIMALS. S.M. Anderson, J.R. Leu, and G.J. Kant. Department of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, D.C. 20307-5100.

The investigation of stress-induced changes in neuronal functioning is important to our understanding of mental disorders, stress-induced psychological impairment, and the emotional reactions of fear and anxiety. Data from previous animal studies have demonstrated various pituitary-adrenal responses to stress and also changes in brain neurotransmitters. We are investigating whether stress-induced neuroendocrine and brain monoamine changes are accompanied by concomitant changes in brain neurotransmitter and/or neuromodulator receptors.

We have developed a behavioral paradigm incorporating sustained stress, continuous performance, and disruption of sleep. Animals which are habituated to press a lever to receive food are trained in an active shock escape task. All animals are housed in operant boxes and receive 45 mg food pellets on a FR 1 schedule. Water is freely available from a drinking tube. Lights are on in the boxes from 0600 to 1800 hrs. After a three day acclimation period, stress trials are presented on a variable time schedule. The average inter-trial interval is ten min. Each trial involves a graduated series of shocks, presented through a metal grid floor. A series consists of 5 seconds at five levels of increasing footshock intensity. Eighty percent of the trials can be terminated at any point during the trial by pulling a chain. Twenty percent of the stress trials are inescapable. A matched set of animals housed in identical operant chambers but not exposed to footshock are used as comparative controls.

After three days of shock exposure, stressed animals and matched controls are decapitated; trunk blood samples are collected; and brains are quickly removed and dissected into discrete regions. All samples are frozen and stored for assay at a later date. Blood plasma corticosterone, prolactin, and adrenocorticotropic hormone are measured as endocrinological indices of stress.

In preliminary experiments we have found that food intake by stressed rats was twenty percent less than that by matched controls. Plasma prolactin and corticosterone levels were higher in stressed rats. [$^3$H]Cyclohexyladenosine (CHA) binding to $A_1$ adenosine receptors in hypothalamic membrane preparations from stressed rats was twenty percent higher than in matched controls. However, no differences in [$^3$H]CHA binding were found in tissue preparations from frontal cortex, hippocampus, or striatum, when comparing stressed and control rats. Additional data comparing neurotransmitter and neuromodulator receptors between stressed and control animals will be presented. Changes in patterns of feeding, drinking, and escape responding over the course of the sustained stress period as well as circadian patterns of these responses will be also be discussed.

223.9 BRAIN ADENOSINE RECEPTORS IN MAUDSLEY REACTIVE AND NON-REACTIVE RATS. P. MONTGOMERY*, E. TAMBORSKA*, P.J. MARANGOS and T.R. INSEL. Unit on Neurochemistry, BPB,NIMH and LCS,NIMH, Bethesda, Md. 20205.

Increasing evidence indicates that adenosine functions as a major non-peptide neuromodulator in brain. The mechanism of the central nervous sytem depressant effects of adenosine probably involves the general inhibition of neurotransmitter release by this purine. In an effort to further characterize the adenosine receptor and its relationship to behavior we investigated receptor status in maudsley reactive (MR) and maudsley non-reactive (MNR) rats. These rats have been selectively bred for high (MR) and low (MNR) degrees of fearfulness. Behavioral testing revealed a significant increase in the amount of defecation and a decrease in exploration by the MR rats. The goal of the present study was to determine whether adenosine or benzodiazepine receptors were altered in the MR rats. The brain areas examined included the cerebral cortex, cerebellum and hippocampus. Three separate studies revealed the most marked changes in the cerebellum with MR rats showing increases in adenosine receptors (using [$^3$H] cyclohexyl-adenosine, [$^3$H]CHA as ligand) ranging from 20-40%. Scatchard analysis clearly revealed an increase in receptor number with no significant alteration in the binding affinity. Examination of benzodiazepine receptors of both peripheral ([$^3$H] RO-5-4864 as ligand) and central type ([$^3$H] ethyl-β-carboline-3-carboxylate as ligand) revealed no significant differences between the MR and MNR strains in either of these sites. This latter finding is in contrast to a previous preliminary report (Europ. J. Pharmacol. 50, 455, 1978).

It, therefore, appears that cerebellar adenosine receptors which are thought to reside on granule cell parallel fibers appear to be increased in MNR rats. The relatively unchanged status of adenosine receptors in other brain areas suggest that cerebellar adenosine receptor increases may be a marker for the reactive trait. Since cerebellar granule cells are thought to use glutamate as their neurotransmitter, it would be interesting to further explore the glutaminergic system in MR rats. It is possible that the biochemical substrate for at least some of the behavioral manifestations of the reactive trait may involve alterations in the cerebellar adenosine system.

223.10 ANTAGONIST-INDUCED OPIATE RECEPTOR UPREGULATION IN CULTURES OF SPINAL CORD AND GANGLION EXPLANTS. A. Tempel, R.S. Zukin and S.M. Crain, Department of Neuroscience, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, NY 10461.

Cultures of fetal mouse spinal cord with attached dorsal root ganglia (DRGs) develop high levels of stereospecific opiate receptors, especially in the DRG neurites (Hiller et al., Brain Res. 1978). Exposure of cord-DRG explants to low levels of morphine (1 uM) for two or more days results in the development of tolerance to the opiates (Crain et al., Life Sci. 1979). We have used this system to study the mechanisms underlying the regulation of opioid receptors by chronic antagonist (naloxone) treatment.

Cord-DRG explants were grown in standard growth medium for two weeks and then in medium containing naloxone (10 uM) for an additional 7 days. Aliquots of explant homogenate (0.25 ml), 0.06 mg protein) were incubated in triplicate with 5 nM $^3$H-dihydromorphine (DHM) in the presence and absence of 10 uM levorphanol. $^3$H-DHM binding to explant homogenates was stereospecific and saturable; half-maximal binding occurred at 3 x $10^{-9}$ M ligand. The time-course of opiate receptor upregulation in the spinal cord-DRG cultures was also examined. Half-maximal receptor upregulation occurred at 2 days of naloxone treatment; receptor density was maximal at 5 days. Assays of cultures exposed to naloxone (10 uM) for 5-7 days revealed a 51% increase in $^3$H-DHM binding relative to that in control cultures. This concentration of antagonist is similar to that required to produce upregulation in CNS tissues by in vivo administration (10 mg/kg/day) (Tempel et al., 1984, 1985). Exposure of the explants to naloxone (10 uM) in the presence of the protein synthesis inhibitor, cycloheximide (1 uM) (a concentration which blocks greater than 90% protein synthesis) resulted in receptor density changes that were similar to those observed in cultures exposed to naloxone alone. This finding suggests that antagonist-induced opioid receptor upregulation does not require the synthesis of new receptor molecules.

In order to determine whether upregulation of this type can occur in the absence of the formation of synaptic connections isolated explants of DRGs or cord were grown in the presence or absence of naloxone. Isolated DRG explants exhibited a more pronounced antagonist-induced increase in receptor number than did DRG-cord explants. This finding indicates that antagonist-induced upregulation of opiate receptors can occur in peripheral DRG neurons in the absence of synaptic connections to their target tissues. It also suggests that regulation of these receptors on isolated DRG neurons may be more plastic. (This work was supported by NIDA Grant No. DA-00069 and NSF Grant No. BNS-8308634 (R.S.Z.) and by NIDA Grant No. DA-02031 (S.M.C.)

223.11 SYNAPTIC INPUT INCREASES ACETYLCHOLINE SENSITIVITY OF EMBRYONIC CHICK SYMPATHETIC NEURONS IN VITRO. L. Role Anat. & Cell Biol. & Ctr. for Neurobiol. & Behav., Columbia Univ. P&S, NY, NY 10032.

The influence of synaptic input on the development of acetylcholine (ACh) sensitivity of sympathetic neurons has been examined using co-cultures of pre- and post-ganglionic neurons from embryonic chick. Preliminary experiments characterize the pharmacology of the transmission and are consistent with an inductive influence of preganglionic input, to increase the peak ACh sensitivity of the neurons following innervation.

Lumbar sympathetic ganglia were dissociated and maintained in vitro for 2-3 days before the addition of dorsal spinal cord explants containing the preganglionic column of Terni. Innervation is established between 24 and 48 hours of co-culture, as evidenced by whole cell recording of spontaneous synaptic currents (PSC's) in neurons voltage clamped near rest potential ($V_H$=-60mV). The PSC's are abolished by addition of 1 um curare to the recording medium and the extrapolated reversal potential is -7mV, the same as for ACh-induced currents (Role, PNAS 81:2924, 1984) consistent with a cholinergic innervation. The amplitude histogram for ~475 synaptic events in one cell was bimodal with peaks at 20 and 40 pA. 1 um tetrodotoxin decreases the frequency of PSC's by 5-25 fold; The remaining PSC's average 23 pA amplitude. Preliminary single channel recording experiments indicate a single amplitude class of ACh channels which, at rest potential, are ~3pA, suggesting that synaptic currents may be comprised of only 6-12 simultaneous channel openings. Therefore, the total number of ACh channels available for synaptic activation might be an important regulatory step in transmission.

To test this possibility neurons grown in the absence of preganglionic input were compared with neurons co-cultured with dorsal spinal cord explants in their development of ACh sensitivity. Neurons were voltage clamped ($V_H$=-60mV) and known concentrations of ACh applied from a nearby pressure ejection pipette. Peak inward current is dependent on ACh concentration, maximal at 100uM and half maximal at 35uM ACh. The slope of the linear portion of the Hill plot (from 2 to 50uM) indicates a Hill coefficient of 1.2 (r= 0.99). Neurons grown in the absence of preganglionic input were relatively insensitive to ACh (100uM; peak current <100 pA, n=9). In contrast peak ACh-currents in innervated neurons of sibling cultures were 4-20 times greater (16 of 18 neurons tested). Neurons in cultures containing explants but without synaptic input were also more sensitive than neurons grown alone (peak current >100-400 pA, n=11). Thus, presynaptic input regulates the transmitter sensitivity of autonomic ganglion cells in vitro. The increase from approximately 10 to >600 ACh channels per cell reflects a dramatic inductive influence of presynaptic input.

223.12 BIOCHEMICAL EFFECTS IN RATS AFTER LONG TERM TREATMENT WITH FLUVOXAMINE AND CLOVOXAMINE: POSTSYNAPTIC CHANGES.

L.D.Bradford, M.Th.M.Tulp* and J.Schipper*. Dept Pharmacoloy, DUPHAR B.V., P.O.Box 2, 1380 AA Weesp, Holland.

Postsynaptic changes after fluvoxamine (FLUV, a specific 5-HT uptake inhibitor), DMI (NA uptake inhibitor) and clovoxamine (CLOV, which has a dual action in blocking reuptake of both NA and 5-HT) were studied after chronic treatment. Administration of FLUV and DMI (20 mg/kg for 21 days) results in a subsensitivity of the β-adrenergic system, as evidenced by a decrease in $^3$(H)-DHA (dihydroalprenolol) binding sites (Bmax) and a reduction of the isoprenaline stimulated adenylate cyclase response (Racagni and Bradford, 1984). Administration of CLOV twice daily (10 mg/kg i.p.) resulted in a decreased cAMP response, but no change in Bmax. In order to determine if the short plasma half life of CLOV in rats could account for the lack of change in Bmax, we compared the sensitivity of the β-adrenergic system after chronic administration of CLOV (t =1hr) and DMI (t =12hr) via twice daily i.p. injections with that via an osmotic minipump in equivalent doses (20 mg/kg/day) for 21 days. Regardless of the dosing scheme, the decrease in the β-receptor coupled response was similar for both compounds. The alteration in β-receptor number did not correlate with the decrease in adenylate cyclase activity. These data support a multistep process of the down regulation of the β-adrenergic system in which the first step is the uncoupling of the receptors resulting in a decrease in the agonist-stimulated adenylate cyclase without necessarily a change in Bmax.

Chronic treatment with all three compounds resulted in a decrease in 5-$HT_2$ ($^3$(H)-Spiroperidol-labeled) receptors in frontal cortex.

Although acutely FLUV has no effect on the β-adrenergic system and DMI no effect on the 5-HT system, adaptational changes on NA and 5-HT systems are observed after chronic treatment with these compounds. These data taken together indicate a functional coupling between the NA and 5-HT systems which may be important in antidepressant mechanisms of action.

Ref: G.Racagni and D.Bradford (1984) Proc. 14th CINP Congress, Florence, Italy: 733.

223.13 PRESYNAPTIC ADAPTATIONAL CHANGES AFTER LONG TERM TREATMENT WITH CLOVOXAMINE. J.Schipper* and L.D.Bradford (Sponsor: P.Bevan). Dept Pharmac., DUPHAR B.V., P.O.Box 2, 1380 AA Weesp, Holland.

There is increasing evidence that longterm treatment with antidepressants induce adaptational changes in pre- and postsynaptically localized transmitter receptors. There up- and down-regulations of transmitter receptors might have important implications for elucidation of the mode of action of antidepressant drugs as well as for the pathophysiology of affective disorders. In this study we evaluated the presynaptic adaptational changes after clovoxamine (CLOV, which blocks NA and 5HT uptake) and desmethylimipramine (DMI, which blocks NA uptake).

CLOV and DMI were administered for 21 days (20 mg/kg/day) either by twice daily i.p. injection or by osmotic minipumps. After a 24 hr washout period, the functional response mediated by alpha$_2$ and 5$HT_1$ receptors was measured using the $^3$(H)-5HT release from cerebral cortex slices in a superfusion system. In slices from control animals the $K^+$ stimulated $^3$(H)-5HT release was inhibited 74±4% by adding 1 μM 5HT to the superfusion medium. In slices from CLOV and DMI treated rats, 71±10% and 73±8% inhibition was found, indicating the absence of 5$HT_1$ autoreceptor subsensitivity. Administration of 1 μM clonidine (alpha$_2$ agonist) resulted in 40±4% inhibition of $K^+$ stimulated 5HT in slices from control animals which was not significantly different in slices from CLOV and DMI treated rats (41±8% and 37±8%, respectively) indicating the absence of subsensitivity of alpha$_2$ receptors. MHPG levels in cerebral cortex were unchanged after longterm DMI and CLOV treatment, indicating an unaffected NA turnover. It was also determined that 5HT, 5HIAA, DA, HVA and DOPAC levels in the striatum were unchanged after treatment, suggesting a normal 5HT and DA turnover. The only observed change after CLOV and DMI was a reduction of 5$HT_2$ receptor binding sites and a down regulation of β receptor coupled adenylate cyclase (Bradford et al, this meeting). Taken together, these data indicate that a functional down regulation of the postsynaptic β adrenergic receptor system is not necessarily paralleled by adaptional changes in presynaptic processes.

224.1 PURIFICATION OF CRAYFISH GAD AND PREPARATION OF MONOCLONAL ANTIBODIES. R. M. Grossfeld and S. A. Hunter*. Zoology Dept., N. C. State Univ., Raleigh, NC 27695-7617.

Glutamic acid decarboxylase (GAD) activity is maintained for 2 weeks in isolated peripheral nerves of crayfish despite the coincident loss of CAT and AChE activities (Sarne et al., Brain Res. 110:91-97, 1976). GAD activity is lost rapidly from these nerves, however, when an inhibitor of protein synthesis is included in the culture medium. Since glial cells are the primary cellular site of protein synthesis in the isolated nerves (Sarne et al., Brain Res. 110:73-89, 1976) Sarne and co-workers have suggested that these cells are a major source of the nerve's GAD.

Our ultimate goal is to determine whether glial cells of crayfish nerve possess GAD immunoreactivity. We have produced monoclonal antibodies using a partially purified crayfish GAD as the antigen. The starting material for preparation of the antigen is the supernatant of an homogenate of crayfish CNS tissue, which possesses about 75% of the total GAD activity and a comparable fraction of the protein; the GAD activity of the homogenate is stable for at least two months despite repeated freezing and thawing. GAD has been partially purified by subjecting the supernatant to conventional gel filtration chromatography on Sephadex G-200 and ion exchange chromatography on DEAE-cellulose. The estimated molecular weight is about 15,000 when GAD is eluted from Sephadex with phosphate buffer containing 2-mercaptoethanol and pyridoxal phosphate.

The partially purified enzyme preparation has been injected into BALB/c mice, which provide spleen cells for generation of colonies of hybridoma cells. Several colonies of cells secrete antibodies which bind strongly to the GAD preparation as revealed by the ELISA technique. We are currently assessing the specificity of the antibodies for GAD using Western blots of the partially purified nerve homogenate after its electrophoresis in non-denaturing gels. (Supported in part by N. C. Agricultural Research Service Project 5582).

224.2 MEMBRANE FLUIDITY CHANGES IN NEUROBLASTOMA CELLS FOLLOWING DIFFERENTIATION BY GANGLIOSIDES OR PROSTAGLANDIN TREATMENT. M.F.D. Notter and J.F. Leary* Departments of Anatomy and Pathology, University of Rochester Medical Center, Rochester, NY 14642.

Neural cell lines have served as model systems to study neuronal development and differentiation in vitro. We have examined changes in membrane fluidity of mouse neuroblastoma cells following differentiation utilizing fluorescent membrane probes and fluorescence polarization. $N_2AB$-1, a subclone of the neuro 2a mouse neural line, was treated with prostaglandin $E_1$/cyclic AMP which induced morphological and biochemical differentiation after two days. Mitotic and differentiated cultures were trypsinized into single cell suspensions and treated with the following membrane conformational probes: 1,6-diphenyl -1,3,5-hexatriene (DPH), a probe of the hydrophobic core of the membrane; 1-(4-(trimethylamino)phenyl) 6-phenyl-1,3,5, hexatriene (TMA-DPH), a probe of the membrane surface; and 8 anilino-1-naphthalene sulfonate (ANS) which occupies the head group interface region of lipid bilayers of membranes and some cytoplasmic structures. These compounds were used at 1 uM, 1 uM and 100 uM respectively. Cells were exposed to each compound for 30 min at 37° and then analyzed for fluorescence intensity and degree of fluorescence polarization at 360 nm at the single cell level by flow cytometry. Upon microscopic examination, it was seen that each probe bound to $N_2AB$-1 cells in a different pattern. DPH appeared in the membrane in a speckled fashion while TMA-DPH outlined the cell completely. ANS stained both cell membrane and cytoplasm. All three probes indicated an increased fluorescence polarization with differentiated cells as compared to mitotic cells. Cells were then treated with 500 ug/ml gangliosides to establish if exogenous glycolipid treatment altered membrane fluidity. Ganglioside treatment induced neuronal differentiation after 24 hrs and stimulated membrane uptake of these compounds as determined by an increase in tetanus toxin binding to live cells by immunofluorescence and cytometric analysis. Upon exposure to the three membrane probes, it was seen that ganglioside treated cells exhibit more fluorescence polarization with ANS and DPH which both partition within the cell membrane. Ganglioside treated cells showed no difference from control cultures when examined with TMA-DPH, a probe of the cell surface. These data indicate that membrane rigidity follows cell differentiation and that this may be due to an increased incorporation of membrane gangliosides.

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224.3 MYO-INOSITOL METABOLISM IN 41A3 NEUROBLASTOMA CELLS: EFFECT OF HIGH GLUCOSE AND SORBITOL LEVELS. M.A. Yorek and B.H. Ginsberg.* Diabetes Endocrinology Research Center, Dept. of Internal Medicine, University of Iowa, Iowa City, IA 52240.

Decreased conduction velocity of peripheral nerves is very common in patients with diabetes. Changes in nerve membrane inositol phospholipids may be responsible at least in part for this problem and in vivo studies have indicated that diabetes may alter inositol metabolism in the peripheral nerve, but the physiological conditions causing this abnormality remain unclear. To ascertain the biological mechanism responsible for this disorder, we have developed a tissue culture system which mimics the altered carbohydrate environment that occurs in diabetes. Neuroblastoma cells were grown in normal conditions as well as in medium supplemented with 30 mM glucose, 30 mM fructose or 1 mM sorbitol. The growth rate of the cells was the same in all four environments as was the total amount of lipid phosphorus/mg of cell protein. Cells grown in the presence of glucose or sorbitol had altered inositol metabolism; both the inositol uptake and the incorporation into phospholipids were decreased by almost 25% in these cells. Fructose supplementation had no effect on either inositol transport or incorporation into phospholipids. Placing cells into glucose supplemented media resulted in an initial small decrease in inositol uptake but a full two weeks of growth in the modified media was necessary to obtain a maximum effect on inositol transport. The effect of glucose and sorbitol was rapidly reversible. After 24 h in normal media inositol transport was restored to control levels. The decrease inositol transport and incorporation into lipids seen with glucose or sorbitol supplementation results in a decrease in the total phosphatidylinositol content within the cell without changing the levels of the other phospholipids. In addition, the uptake of choline, serine or ethanolamine was unaffected by the supplemental conditions. Because the presence of 30 mM fructose had no detrimental effect on the cell metabolism, the reduced inositol uptake observed in this system is probably not due to a change in the osmolarity of the medium. Although glucose may be partially reducing inositol uptake by competing for transport, the time course of the effect implies a more complex mechanism is involved. It is possible that sorbitol or an unidentified intermediate common to both glucose and sorbitol may be responsible for this effect or that glucose or sorbitol may be inducing a membrane change resulting in a suppression of inositol transport. These results suggest that the high circulatory levels of glucose and sorbitol which exist in unregulated diabetes may be partially responsible for some diabetic neuropathy. (Supported by NIH Grant AM25295.)

224.4 FODRIN: SKELETAL PROTEIN CROSS-LINKER IN RAT BRAIN SUBCELLULAR FRACTIONS. Robert Siman and Gary Lynch, Department of Psychobiology, Univ. of California, Irvine, Ca. 92717.

Fodrin is a major protein of mammalian neurons and is a variant of spectrin, a primary component of the cytoskeleton of erythrocytes. While fodrin has been hypothesized to link cytoplasmic actin filaments to neuronal membranes, there is no direct evidence for fodrin-cytoskeletal interaction. We have investigated this issue by immunoprecipitating fodrin from rat brain subcellular fractions with specific antibodies. The fodrin-antifodrin complexes were collected using protein A-bearing Staph. aureus, and the polypeptides co-precipitating with fodrin were identified by gel electrophoresis, peptide mapping, and Western blotting.

In crude microsomal (S2) fractions from cerebral cortex fodrin immunoprecipitates contained, in addition to the Mr= 240,000 fodrin subunits, polypeptides of Mr=300,000, 200,000, 180,000 and 46,000. The first was identified as microtubule-associated protein 1 (MAP1), the last as actin. The identity of the 200,000 and 180,000 polypeptides is currently under investigation. In S2 fractions from brainstem, polypeptides of Mr=300,000, 210,000, 160,000 and 70,000 co-precipitated with fodrin. The former was identified as MAP1, the latter three as the neurofilament triplet. These proteins were not directly recognized by fodrin antibodies, but rather were precipitated as a consequence of their association with fodrin.

To identify proteins associated with membrane-bound fodrin, the fodrin was extracted with low or high ionic strength buffer and immunoprecipitated as before. With either extraction method cortical fodrin co-precipitated with MAP1, MAP2 (Mr=270,000), and actin, while brainstem fodrin co-precipitated with MAP1, actin, and the neurofilament triplet. The fodrin-neurofilament interaction occurred via binding of fodrin to the Mr=210,000 polypeptide. These data demonstrate that fodrin binds to protein components of all three cytoskeletal systems of neurons. Fodrin may play a fundamental role in mediating cytoskeletal-membrane interactions in neurons, and in organizing skeletal proteins for their transit in the slow phase of axoplasmic transport.

Supported by grants from NSF (G.L.) and the Hereditary Disease Foundation (R.S.).

224.5 STRUCTURE OF THE AXON HILLOCK AND INITIAL SEGMENT OF FROG DORSAL ROOT GANGLION CELLS. E. Matsumoto* and J. Rosenbluth (SPON: B. Pachter). Departments of Physiology and Rehab. Medicine, New York University School of Medicine, New York, NY 10016

The plasmalemma of the axon hillock (AH) and initial segment (IS) of frog dorsal root ganglion cells and the structure of the overlying satellite cells were examined in freeze-fracture replicas and thin sections. The concentration of P-face particles in the neuronal plasmalemma at the AH (~ 2500/μ2) approximates that in the cell body (CB) and IS membranes (~ 2000/μ2). In contrast, the E-face particle concentrations in the respective regions are strikingly different. The plasmalemma of the CB contains ~ 300 particles/μ2, while that of the AH contains ~ 1000/μ2 and that of the IS ~ 800/μ2. The particle concentrations in the latter two regions approach that at the node of Ranvier, and, moreover, particle size analysis reveals that these particles, like those at the node, include a high proportion (~35%) that are large (≥10nm). In contrast to the uneven particle distribution in the neuronal plasmalemma, particle concentrations in the satellite cell membrane are similar in all three regions (P-face, ~ 1500/μ2; E-face, ~ 1000/μ2). The gross structure of the sheath, however, varies considerably. Around the CB the sheath is composed of one to several thin satellite cell lamellae closely applied to the neuronal plasmalemma. The innermost layer is separated from the neuronal plasmalemma by ~ 10-50nm, and the outermost layer is covered by a basal lamina. At the AH, in contrast, the sheath is split into outer and inner components separated by a broad extracellular space, distinct from the endoneurium, into which trabecular processes of the satellite cells project. These processes are surrounded by a flocculent material similar to the matrix surrounding the node of Ranvier. The split sheath continues over the proximal portion of the IS to varying degrees, extending ~ 5 μ to more than 20 μ from the CB. Externally, the basal lamina of the CB sheath continues over the proximal IS sheath and onto the distal portion. In contrast to the CB sheath, that around the IS is very irregular. In some regions it consists of as many as 50 lamellae. In others, it exhibits node-like interruptions. Patches of dense axolemmal undercoating are also present in this region. Distally, the IS terminates at a heminode, where the satellite cell and the adjacent Schwann cell both extend multiple villous projections toward the axon over a length of ~ 1-3 μ. The axolemma in this region is undercoated as well. It is concluded from these results that the AH and IS of frog dorsal root ganglion cells and the satellite cell sheath surrounding the AH and IS have some of the structural characteristics of the axon and Schwann cell sheath at the node of Ranvier, suggesting functional similarities between the respective regions. Supported by grants from the NIH (NS 07495) and Muscular Dystrophy Association.

224.6 EFFECTS OF OLIGODENDROGLIAL DEFICIENCY ON DEVELOPING AXONS AND AXOLEMMA OF RAT OPTIC NERVE. J.A. Black, M.D. Feliciano*, S.G. Waxman, and B.R. Ransom. Dept of Neurology, Stanford Univ. Sch. of Med., V.A. Med. Ctr. Palo Alto, CA 94305.

The normal sequence of gliogenesis in the rat optic nerve was disrupted by neonatal treatment with 5-azacytidine (5-AZ). This protocol markedly reduced the number of glial cells, especially oligodendrocytes, and myelin sheaths observed in 15 day old rats. Thus, the structure of developing axons and axolemma may be studied in the absence of normal axo-glia interactions.

Rat pups were given subcutaneous injections of 5-AZ at a dosage of 3.3 mg/kg. 5-AZ was dissolved in PBS at a concentration of 3 mg/ml. Injections commenced the day following birth, and were given once daily until 15 days of age.

Control and 5-AZ-treated rats were perfused with 2% glutaraldehyde and 2% paraformaldehyde in 0.14 M phosphate buffer containing 0.1 M sucrose, and was processed by conventional methods.

Morphometric analysis of optic nerves from 2 and 15 day control and 15 day 5-AZ rats was performed. The values are mean ±S.D.

| | 2 d cont. | 14 d cont. | 14 d 5-AZ |
|---|---|---|---|
| | | Optic nerve diameter (μm) | |
| | 186.0±15.3 | 346.4±17.3 | 302.7±38.5 |
| | | Volume density (%) | |
| unmyel. | 53.0±8.0 | 29.2±6.6 | 46.9±8.4 |
| myel. | 0. | 27.0±7.0 | 0.1±0.1 |
| glia | 32.0±9.9 | 36.6±8.4 | 50.7±8.5 |
| e.c.s. | 13.0±3.6 | 6.0±3.1 | 1.6±1.1 |
| bl. v. | 1.0±0.5 | 0.9±2.6 | 0.7±2.2 |
| | | Glial cells/1000μm$^2$ | |
| | 4.3±0.7 | 2.8±0.3 | 3.1±0.4 |
| | | Axonal diameter (μm) | |
| unmyel. | 0.22±0.04 | 0.37±0.09 | 0.38±0.09 |
| ensheath. | 0. | 0.53±0.11 | 0.63±0.15 |
| myelin. | 0. | 0.77±0.18 | 0.80±0.08 |

Analysis of freeze-fracture replicas of optic nerve fibers from control and 5-AZ-treated rats reveal the following intramembranous particle (IMP) densities (mean±SEM):

| Condition | P-face (IMP/μm$^2$) |
|---|---|
| 2 d control unmyel. | 512±50.7 |
| 15 d control unmyel. | 431±17.4 |
| 15 d control internode | 1010±74.1 |
| 15 d 5-AZ (<0.5 dia.) | 651±62.2 |
| 15 d 5-AZ (>0.5 dia.) | 917±34.9 |

These data suggest that gliogenesis in the rat optic nerve may be suppressed by treatment with 5-AZ, and that axonal and axolemmal differentiation may occur in the absence of normal glial interactions. (Supported by NIH NS-15320, NMSS RG-1231 and VA Med. Res. Svc.)

224.7 AN UNUSUAL MEMBRANE SPECIALIZATION IN PRIMARY CULTURE OF THE RAT CEREBRAL CORTEX. J.-H. Tao-Cheng and M. W. Brightman*, Laboratory of Neurobiology, NINCDS, NIH, Bethesda, Maryland 20205

Cerebral cortex from 2 day to 4 week old rats were cultured for 8 to 23 days and processed for freeze-fracture electron microscopy to study their membrane structure. We have found fairly frequently an unusual membrane specialization which consists of parallel rows of particles in both P and E fracture faces. The rows form a slight prominence on the P face and a shallow depression on the E face. The size of the particles and the distance between the particles vary. The length of the rows is usually about 100nm but there are a few that are smaller. The interval between rows is fairly constant at about 35nm. The rows may form a column of 3-10 rows, with 5-6 being the most common. Instead of columns, some rows form an arc or a complete circle where the rows radiate from a particle-free center. In these cases, the total number of rows can reach 30-40. Caveolae usually lie near the periphery of the rows, and the E face has an unusually high density of background particles. The only other specialization within the same membrane is the gap junction. Since no assemblies, tight junctions or characteristic elongated particles are found in the same membrane as the particle rows, the cells are probably neither fully expressed astrocytes nor differentiated oligodendrocytes.

The particle rows resemble the "ciliary necklace" of ependymal cells in vivo in the regular periodicity of the rows, in the size and spacing of the particles, but not in the configuration of the rows. Small patches of similar particle rows have been described in the cell membranes of developing ependymal cells in vivo from 17-21 day fetal rats but not in older animals. If the particle rows are ciliary, they appear in the plasma membrane first, then either migrate or are carried into the ciliary membrane as the cilium begins to elongate.

Although the particle rows in vivo are a feature of immature ependymal cells, it is unlikely that the particle rows in vitro belong to donor ependymal cells that had dedifferentiated because (1) ependymal cells only comprise a very small proportion of cells in primary brain cultures, and (2) the occurrence of the particle rows in our cultures is too frequent to be accounted for by the few ependymal cells. Thus, the particle rows in vitro are probably expressed by a type of non-ependymal glial cell which is most likely in immature forms.

224.8 THE DIFFERENTIATION OF MEMBRANE STRUCTURE IN CULTURED ASTROCYTES. D.M.D. Landis, L.A. Weinstein, and E.L. Coe. Neurology Service, Massachusetts General Hospital, Boston, MA 02114

Assemblies of orthogonally-packed, uniform intramembrane particles are highly concentrated in astrocytic membranes investing blood vessels and forming the surface of the brain, and are less common in astrocytic processes adjacent to neuronal membranes. Astrocytes in primary culture also can acquire assemblies in the presence of certain media, but the assemblies tend to be more uniformly distributed across the cell surface. We have examined the influence of various tissue culture media and substrates on the density of assemblies, their distribution over the cell, the proportion of cells expressing them, and the rate of cell proliferation.

Astrocytes were obtained from the forebrains of 1-2 day rat pups with a combination of mincing and enzymatic digestion, plated in 75cm$^2$ flasks, and fed with new L15 medium plus 20% fetal calf serum at 3 day intervals for 7-10 days until confluent. The cells were re-plated in petri dishes, using methods slightly modified from those of McCarthy and deVellis, and the culture conditions then systematically altered. The rate of growth was estimated by counting cells in a selected field, the proportion of astrocytes estimated by determining the number of cells with glial fibrillary acidic protein, and membrane structure was examined after aldehyde fixation.

The density of assemblies and the proportion of cells expressing assemblies were coordinately affected by the lot of fetal calf serum used to supplement the media. With the most effective fetal calf serum, 89% of the cultured cells manifested assemblies, with a mean density of 20.17/sq micron. With the least effective lot, 62% of cells expressed assemblies, with a mean density of 2.08/sq micron. Addition of glucocorticoid or alteration of potassium concentration in the medium had little effect. The proportion of GFAP+ cells did not vary appreciably.

Although astrocytic membranes adjacent basal lamina in vivo have the highest concentrations of assemblies, culture of astrocytes on a collagen substrate was essentially similar to culture on the plastic alone or on a poly-lysine substrate. Astrocytes grew very rapidly on basal lamina laid down by primary cultures of bovine cerebral endothelial cells, but there was no change in assembly density or distribution.

Various fractions of fetal calf serum are being compared to determine the approximate molecular weight of the component(s) affecting assembly density in cultured astrocytes.

224.9 ANOXIA DOES NOT ALTER MEMBRANE STRUCTURE IN CULTURED ASTROCYTES. L.A. Weinstein and D.M.D.Landis. Neurology Service, Massachusetts General Hospital, Boston, MA, 02114. (SPON: J.J. Halperin)

Freeze-fracture studies of the mammalian nervous system have revealed large numbers of "assemblies", orthogonally-packed aggregates of uniform particles, in the membranes of the astrocytic processes investing blood vessels and forming the brain surface. Astrocytic processes surrounding neuronal structures have fewer assemblies. The function of this specialization of intramembrane particle distribution is unknown, but its concentration at the brain's interface with blood and cerebrospinal fluid suggests a capacity for transport across that interface.

In cerebellar cortex rapidly frozen within 35 seconds of circulatory arrest, the structure of assemblies in the glial limitans is altered, and in tissue rapidly frozen at longer intervals after decapitation there is a progressive disappearance of the particle arrays (J Cell Biol. 88:660, 1982). By contrast, membrane structure in neuronal processes is little changed over the same intervals. The selective vulnerability of astrocytic membrane structure parallels the emergence of swelling in the same processes.

In order to determine whether anoxia per se or some other sequela of circulatory arrest caused the change in membrane structure, we have used the more easily controlled system of primary astrocyte cultures. Astrocytes were obtained from 1-2 day rat pups using methods slightly modified from McCarthy and deVellis; 70-90% of the cultured cells are astrocytic as judged by glial fibrillary acidic protein immunoreactivity. A slightly smaller proportion have assemblies distributed fairly uniformly over the cell surface. Cultures were exposed to 95% nitrogen 5% $CO_2$ or 100% nitrogen (depending on the culture medium buffer system) and fixed with aldehydes after various intervals up to 30 minutes; some cultures were allowed to 'recover' in 95% oxygen and were then fixed. The fixed cultures were fractured, and the number of assemblies in their membranes was counted. We did not observe any change in assembly structure or any change in the range of assembly densities in the anoxic cultures.

We suspect that anoxia in isolation is insufficient to cause the changes previously observed in the astrocytic membranes of cerebellar cortex, and that changes in pH or potassium concentration may also have a role. It is also possible that aldehyde fixation is inadequate to detect the membrane alterations revealed by rapid freezing methods.

## NEURAL PLASTICITY IN ADULT ANIMALS II

225.1 AN ANALYSIS OF SYNAPTIC TRANSMISSION IN THE TRISYNAPTIC PATHWAY OF THE HIPPOCAMPAL FORMATION DURING KINDLING OF THE PERFORANT PATH. T. Sutula, O. Steward, Dept. of Neurology, Univ. of Wisconsin, Madison, WI 53792, and Depts. of Neurosurgery and Physiology, Univ. of Virginia, Charlottesville, VA 22908

Although kindling induces transsynaptic alterations (Messenheimer, et al., Exp. Neur., 1979), the nature of the transsynaptic alterations and the identity of pathways altered during kindling are uncertain. Kindling of the entorhinal cortex (EC) induces an increase in synaptic efficacy similar to long-term potentiation (LTP) in the monosynaptic pathway to the dentate gyrus (DG) (Douglas, Goddard, Brain Res., 1975), (Sutula, Steward, Neurol. 33 (2), 188, 1983). However, recent studies have shown that the DG is not necessary either for the development or maintenance of EC kindling. (Harrison, et al., Soc. Neurosci. Abstr. 10 (1), 346, 1984). Furthermore, repetitive induction of LTP at EC-DG synapses is not sufficient to induce kindling, but facilitates subsequent kindling by EC stimulation (Sutula, Steward, Soc. Neurosci. Abstr. 10 (1), 78, 1984). These observations indicate that LTP at EC-DG synapses cannot be the entirety of the mechanism of kindling, and direct attention to possible alterations in multisynaptic pathways as the underlying mechanism of kindling.

To evaluate the hypothesis that behavioral evolution of kindled seizures proceeds with progressive spread of evoked activity in multisynaptic pathways, the EC was stimulated, and evoked responses were recorded from the hilus of DG and the stratum lacunosum-moleculare of CA1. Input-output relationships were recorded in the monosynaptic pathway (EC-DG) and trisynaptic pathway (EC-CA1) prior to and during evolution of kindled seizures. A single kindling stimulus which evoked AD induced synaptic potentiation in the EC-DG pathway, and the smooth contoured negativity recorded at a latency of 10-12 msec from CA1 (Andersen, et al., Exp. Brain Res. 1966) was replaced by a sharp contoured negativity followed by a high amplitude positivity at 12-14 msec. The alterations in evoked responses recorded from the DG and CA1 developed after one afterdischarge, and persisted through class V seizures.

Increases in synaptic efficacy in multisynaptic pathways of the hippocampal formation occur early in the course of kindling, when behavioral seizures are minimal. The results direct attention to a possible role for synaptic potentiation in the emergence of epileptic activity in multisynaptic neuronal networks. Further study is necessary to define the cellular basis of the alterations in multisynaptic transmission and to determine whether synaptic potentiation develops at each link of the trisynaptic pathway during kindling.

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225.2 LONG-TERM POTENTIATION IN LIZARD CEREBRAL CORTEX. J.R. Larson* and G. Lynch (SPON: H.D. Schwark). Center for the Neurobiology of Learning and Memory, Univ. Calif., Irvine, CA 92717.

Long-term potentiation (LTP) of excitatory postsynaptic potentials (EPSPs) following brief periods of high frequency synaptic activation is a prominent feature of mammalian hippocampus. Comparisons of this effect with synaptic plasticities found in more "primitive" cortices of non-mammalian vertebrates may yield important clues about the nature of its cellular substrates. Toward this end, we have developed a new in vitro preparation of reptilian cortex, a structure that bears striking anatomical and histochemical similarities to mammalian hippocampus.

Experiments were performed on desert iguanas (Dipsosaurus dorsalis). The entire cerebral cortex of each hemisphere was isolated and maintained in vitro. Stimulation of lateral (LC) or dorsal cortex (DC) or the pial surface of dorsomedial cortex evoked a short-latency monophasic negative field potential recorded at the pial surface of the medial cortex (MC). Laminar profiles suggested that the response reflected monosynaptic EPSPs generated by LC and/or DC afferents to apical dendrites of MC neurons.

Initial slope and peak amplitude of evoked responses were measured at 10-60 sec intervals for 10-60 min before and 20-300 min after high frequency stimulation (HFS) which consisted of 3-5 trains of 50-100 pulses at 100 Hz. HFS typically induced post-tetanic potentiation (PTP) which decayed within 5 min and LTP which decayed very little thereafter. In 18 of 20 experiments, the slope was at least 110% of control 20 min after HFS (mean ± s.e.m. of 143±5%). In five experiments, mean potentiation was 146% (±16%) 2 hr after HFS. The majority of experiments were performed at 35-40°C but LTP could also be induced at room temperature (24°C).

Experiments using two stimulation electrodes evoking responses in the same population of postsynaptic neurons indicated that the LTP effect was restricted to synapses activated at high frequency.

In contrast to the the reliable LTP seen in apical dendritic synapses of MC neurons, HFS of afferents to basal dendrites of the same neurons did not induce LTP (mean slope of 98±4% of control 15 min after HFS in ten experiments).

These experiments indicate that the in vitro reptilian cortex offers a stable and, compared to brain slices, relatively undamaged preparation that is well-suited for neurophysiological experiments. Moreover, it is clear that the reptilian cortex exhibits a form of synaptic plasticity similar, if not identical, to LTP but that the effect is not as widespread as is the case in mammalian hippocampus. Tests for biochemical and anatomical differences between the projections to the apical and basal dendrites of the MC neurons as well as between these and hippocampal synapses may provide suggestions about the substrates of LTP.

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225.3 LONG-TERM POTENTIATION IN THE IN VITRO RAT HIPPOCAMPUS. P.G. DiScenna & T.J. Teyler, Program in Neurobiology, Northeastern Ohio Universities College of Medicine, Rootstown, OH. 44272.

We are presently working on three questions regarding the induction of LTP in area CA1 (Alger & Teyler, 1976; Schwartzkroin & Wester,1975). 1) Why is LTP so capricious? Given the standardization of procedures within a lab, why is the reliability of LTP induction often significantly less than 100%? 2)What, if any is the stimulus threshold for LTP in CA1? While there is evidence to suggest that higher intensity stimulation produces LTP more reliably (Lee,1983; Schwartzkroin & Wester, 1975), recent studies suggest that long-term changes can be elicited with intensities well below spike-threshold (Barrionuevo & Brown, 1983). 3) Is associative LTP limited to neighboring terminal patches or can this phenomenon be produced with inputs that are spatially distinct?

To these ends we are accumulating an internal data base as a control with which to compare the results of experiments aimed at answering these questions. 400um. slices of adult rat hippocampi were obtained using standard slice procedures and maintained in media containing 124mM NaCl, 3.3mM KCl, 2.0mM Ca, 2.0mM Mg, 10 mM glucose and 26mM bicarb. All experiments were run at least 90 min. after slicing. Stimulus intensity was set to elicit a field potential (recorded from the alvear side of the pyramidal cell layer) with a 0-2mv. spike driven from the stratum radiatum. The stimulating electrodes are 75um. tungston wire, sputter-coated with gold to produce a thin bipolar electrode which produces a near-negligible stimulus artefact (Chiaia & Teyler, 1982). The tetanus consisted of three bursts (200ms.) at 200Hz. delivered at 10 sec. intervals.

The initial analyses of 116 experiments (62 experiments monitored for 30 min. and an additional 54 monitored for 60 min.), demonstrated that 93 ( approximately 80%) developed increased synaptic efficacy relative to a 10 min. pre-tetanus baseline (eg. 100-1000% increase in population spike amplitude). PTP, which usually peaked at 500-1000% above baseline, was observed in all experiments with the exception of three cases (These three, however did demonstrate LTP). The decaying effect of PTP and the rising effect of LTP intersect during the 2-5 min. interval post-tetanus.

225.4 A CURRENT-SOURCE DENSITY ANALYSIS OF HIPPOCAMPAL CA1 REGION FOLLOWING LTP. J.S. Taube and P.A. Schwartzkroin. Dept. of Neurological Surgery, University of Washington, Seattle, WA 98195.

One puzzling observation concerning long-term potentiation (LTP) is that the population spike is potentiated more than expected given the potentiation of the population EPSP. An explanation for this differential potentiation is that there is some alteration in the distribution of current sinks or sources. Changes in the density and/or location of dendritic channels could lead to altered sites or thresholds for spike initiation. To test this hypothesis, we performed a current-source density analysis in the CA1 area of the hippocampus before and after a LTP-inducing tetanization.

Guinea pig hippocampal slices (400 um thick) were prepared and maintained in vitro at 35° C. Three extracellular microelectrodes spaced 30 um apart were aligned perpendicular to the CA1 pyramidal cell layer. The three electrodes were moved along a track perpendicular to pyramidale, at intervals of 30 um, from str. moleculare to str. oriens (s.o.). Bipolar stimulating electrodes were placed 200-300 um into str. radiatum (s.r.) to activate the Schaffer collaterals. Six responses were recorded at each point before and after a 100 Hz, 1 sec. stimulus train. The extracellular recordings were analyzed according to the methods of Freeman and Nicholson (J. Neurophysiol. 38:369-382, 1975).

For each experiment, 4 time points were chosen for analysis based on the waveform generated in pyramidale: 1) At a time point before the first positivity, a weak sink sometimes developed following LTP in proximal apical dendrites. 2) During the peak of the first positivity (pop. EPSP), a stronger sink developed in s.r. that varied in distance (0-100 um) from pyramidale. No movement of this sink was detected post-tetanization. A source was also consistently evident between pyramidale and 60 um into s.o. Both sink and source increased in intensity following LTP. 3) The time of the peak negativity (pop. spike) showed the most dramatic changes. Starting in s.r. and going toward s.o., a pattern of sink followed by source was initially evident. Following LTP, a second sink developed and the pattern became sink-source-sink. This pattern varied in location along the neuron axis, but usually began between 0-100 um into s.r. The peak of the source was relatively localized; after tetanization, it moved apically. 4) During the peak of the second positivity (pop. IPSP?), a source was present near pyramidale which grew in intensity following LTP.

These results are consistent with the idea that a stable dendritic sink is potentiated following LTP (i.e., EPSP potentiation). In addition, a novel sink develops post-tetanization, suggesting the induction of a new active zone in these cells and providing a possible mechanism for EPSP-spike dissociation. Finally, it appears that IPSPs are also potentiated by the tetanization.

These studies were supported by NIH grants NS18895 and GM07108.

225.5 CHANGES IN SYNAPSE DENSITY CORRELATE WITH LONG-TERM POTENTIATION IN THE DENTATE GYRUS AT SHORT SURVIVAL INTERVALS. N. L Desmond and W. B Levy. Dept. Neurosurg., Univ. of Virginia School of Medicine, Charlottesville, VA 22908.

Long-term potentiation (LTP) of the entorhinal cortex-dentate granule cell dendritic spine synapses correlates with several morphological changes 10 and 60 min following a spaced conditioning stimulation paradigm[1,2]. Here we report that these same structural correlates exist at shorter survival intervals following brief, high-frequency conditioning stimulation that induces LTP. Conditioning stimulation (24 8 pulse 400 Hz trains, 1 train/5 s) was delivered to one angular bundle. Animals were sacrificed 10 min (N=6) or immediately (2 min group, N=6) following the 24th train. Animals were perfused with mixed aldehydes. Blocks of dentate gyrus were prepared[1] for electron microscopy. Asymmetric shaft and spine synapses were identified double-blind on montages of the dorsal leaf molecular layer. Spine synapses were categorized by shape[1], and these counts were converted stereologically to number of synapses per unit volume of tissue ($N_V$). The middle third of the molecular layer is the region of primary synaptic activation during conditioning stimulation[1]. The $N_V$ for the entire molecular layer is unchanged at both survival intervals following conditioning stimulation, with control $N_V$ values of 2.44±0.09 and 2.32±0.18 synapses/$\mu m^3$ for the 10 and 2 min groups, respectively (mean±sem). In the middle molecular layer, $N_V$ decreases overall with potentiation (10 min group: 4.5% decline from control $N_V$ of 2.57±0.15, NS; 2 min group: 12.1% decline from control $N_V$ of 2.56±0.16, t=-3.063, $p<0.01$). At 10 min, the $N_V$ for concave spine synapses increases 35% from the control $N_V$ of 0.537 synapses/$\mu m^3$ in the middle molecular layer (t=2.108, $p<0.05$) while at 2 min the $N_V$ for concave spine synapses increases 15% over the control $N_V$ of 0.604 synapses/$\mu m^3$ (NS) there. The increased density of concave spine profiles in the middle molecular layer is accompanied by significant decreases in the density of non-concave (simple and ellipsoid) spine profiles there. At 10 min, the $N_V$ for non-concave spine profiles decreases 16.6% from a control $N_V$ of 1.820 synapses/$\mu m^3$ (t=-1.708, $p<0.05$). At 2 min, the $N_V$ for non-concave spine profiles declines 23.0% from a control $N_V$ of 1.757 synapses/$\mu m^3$ (t=-2.018, $p<0.05$). These data extend previously reported changes in spine synapses with LTP[1,2] and add further support to our[2] hypothesis of an interconversion of spine synapses with LTP. The absence of a marked time course of alterations in synapse density with LTP suggests that the interconversion of spine synapses from non-concave to concave occurs rapidly following the induction of potentiation and persists for at least 60 min after conditioning stimulation.

Supported by NS15488 and AFOSR 83-0236 to WBL. [1]Desmond & Levy. Brain Res. 265(1983)21. [2]Levy & Desmond. Anat. Rec. 211(1985)111A.

225.6 ULTRASTRUCTURAL IDENTIFICATION OF SYNAPSES OF THE CROSSED TEMPORO-DENTATE PATHWAY OF THE RAT. O. Steward and L. Davis*. Dept. of Neurosurgery, and the Neuroscience Program. Univ. of Virginia, School of Med., Charlottesville, VA 22908.

The crossed temporo-dentate pathway (CTD) from the entorhinal cortex (EC) to the dentate gyrus (DG) has proved to be an excellent site to investigate various types of synaptic plasticity, including long-term potentiation (Levy and Steward, Brain Res., 1979, 175: 233-245, and Neuroscience, 1983, 8: 791-797) and lesion-induced synapse growth. The structural features of CTD synapses have remained obscure, however, since electron microscopic studies have failed to reveal degenerating synapses in the contralateral DG after EC lesions. A clue to why this might be the case is that terminal degeneration of the CTD (as revealed by Fink-Heimer staining) disappears rapidly after lesions (i.e., by 5 days) (Goldowitz, et al., Exp. Neurol., 1975, 47: 433-441). In this regard, it is of interest that the ipsilateral temporo-dentate pathway exhibits two forms of degeneration: the dark dense variety, which is long lasting, and a lucent variety which disappears rapidly (Fifkova, Brain Res., 1975, 96: 169-175, Steward and Vinsant, J. Comp. Neur., 1983, 214: 370-386). It was thus of interest to ask whether the degeneration in the CTD might be of the electron lucent type.

The EC was destroyed unilaterally in adult male Sprague-Dawley rats. Forty-eight hours after the lesion the rats were perfused transcardially with 2% glutaraldehyde/2% paraformaldehyde in cacodylate buffer, and sections of the contralateral DG were prepared for routine electron microscopy. The middle molecular layer of the DG was scanned at high magnification for degenerating profiles.

Synapses exhibiting what appeared to be the electron lucent form of degeneration were observed in the middle molecular layer of the DG at 2 days post lesion. The electron lucent profiles could be demonstrated to be synapses because of the presence of a postsynaptic density. A few dark dense profiles were also observed, but none of the dark dense profiles appeared to make synaptic contacts, thus confirming previous reports of an absence of dark dense degenerating synapses in the contralateral DG after EC lesions. In general, the synapses appeared comparable to those of the ipsilateral temporo-dentate system, in that they terminated on spines. The results suggest that the CTD pathway undergoes exclusively the electron lucent form of degeneration, which accounts for the rapid disappearance of Fink-Heimer degeneration products at the light microscopic level. The reason for the lack of the electron dense form of degeneration is not clear. (Supported by NIH 12333 to OS).

225.7 TIME COURSE OF EMERGENCE OF LTP DURING REINNERVATION OF THE DENTATE GYRUS. T. Reeves, & O. Steward , Neurosurg. Dept., Univ. Virginia Med. Sch., Charlottesville, VA 22908

A current hypothesis regarding the mechanism of LTP is that the increase in physiological potency results from changes in spine shape, which alter the resistance of spines to current flow (Crick, 1982, Trends Neurosci., 5, 44-46; Rall & Rinzel, 1973, Biophys. J., 13, 648-688). One approach to testing this model is to measure the capacity for LTP in fiber pathways undergoing lesion-induced sprouting, where presynaptic structures form on spines of highly abnormal shape early in the reinnervation period. The crossed temporo-dentate (CTD) pathway does not normally exhibit LTP. However, following unilateral entorhinal cortex (EC) lesions the remaining CTD system sprouts, and at long post-lesion intervals will support LTP. This situation is of considerable interest because the synapses begin to operate early in the course of sprouting (Reeves, Smith & Jensen, 1983, Neurosci. Abstr., 9, 985), but at this time they contact abnormally stubby spines that lack narrow necks. If LTP is mediated through a regulation of spine shape one would predict that LTP would be severely disrupted early in the course of reinnervation.

Adult Sprague-Dawley rats received unilateral EC lesions, and survived for various post-lesion intervals (6 to 40 days). Rats were then anesthetized with chloralose-urethane and prepared for acute electrophysiological recording. A bipolar stimulating electrode was positioned in the intact EC, and glass microelectrodes were lowered into the dentate hilus of both hemispheres. Baseline evoked potentials were measured at a test frequency of 0.1 Hz, and then 8 high-frequency trains (400 Hz; duration 20 msec/train) were delivered. Field potentials were again evoked at the test frequency. The CTD system evidenced clear potentiation of the population EPSP as early as 8 days post-lesion (5-10% response increases); the capacity for LTP increased after 8 days, and reached an asymptotic level at around 16 days post-lesion (15-20% response increases). The ipsilateral temporo-dentate pathway, recorded simultaneously, showed similar increases (mean of 22.11%).

The capacity for LTP emerged between 8 and 12 days post-lesion, at a time when spines on the postsynaptic cells are structurally immature (Caceres & Steward, 1983, J.Comp.Neurol., 214 , 387-404; Steward, 1983, J. Neurosci., 3, 177-188). It seems doubtful that such spines would significantly attenuate current flow, and thus changes in spine shape would be unlikely to result in altered current injection into the dendrites. These results suggest that LTP can occur through some mechanism(s) other than changes in spine shape. Supported by NSF grant BNS-8021865-02 (O.S.) and NRSA fellowship NS-07199 (T.R.).

225.8 ALTERED SYNAPTIC REORGANIZATION, FOLLOWING PARTIAL DEAFFERENTATION OF THE RAT DENTATE GYRUS, DURING SHORT-TERM ETHANOL EXPOSURE. E. Orona, B. E. Hunter, and D. W. Walker. Veterans Administration Medical Center, Dept. Neuroscience and Alcohol Research Center, University of Florida College of Medicine, Gainesville, FL 32610.

The phenomenon of reactive synaptogenesis in the dentate gyrus following partial deafferentation was used to quantitatively assess the effects of short-term ethanol exposure. Two groups of adult male Long-Evans rats received unilateral electrolytic lesions of the entorhinal cortex. For the next 40 days, the rats were maintained on nutritionally complete ethanol- or sucrose-containing liquid diets. Horizontal 40 micron frozen brain sections were cut and processed for acetylcholinesterase (AChE) staining. The bandwidth and optical densities of afferent terminal fields were measured in the dentate gyrus using a computer based image analysis system (EyeCom II/DEC 11/23). Reorganization of the commissural/associational (C/A) and cholinergic (primarily septal) afferents was evaluated from 10 x 10 arrays of measurements in each blade of the lesioned and unlesioned dentate gyrus in each animal. These 10 x 10 data arrays consisted of measurements, perpendicular to the molecular layer (ML), made automatically at 10 equally-spaced positions along each blade in each of 10 equally-spaced horizontal sections.

Entorhinal lesion alone produced a 14% decrease in total ML width on the ipsilateral side, compared to the contralateral side; whereas ethanol treatment alone produced a 3% decrease in ML width. The lesion-induced expansion in the bandwidth of the C/A zone was reduced by about 50% in the ethanol, as compared to the control group. There was also less "clearing" of the C/A zone following the lesion in the ethanol animals, in terms of density of AChE staining. In both buried and exposed blades, the lesion-induced effects in the ethanol group were greater dorsally than ventrally. This was true although the ethanol group had generally lighter overall AChE staining than the control group, and although the staining was more intense dorsally than ventrally for both groups. In addition to the differences in the C/A zone on the lesioned side, the ethanol group had a greater "condensation" of AChE reaction-product in the outer molecular layer than did the control group.

These results coupled with previous data (West et al., Science, 218: 808-810, 1982) indicate that there is a significant alteration in the capacity for synaptic reorganization in the adult CNS following short-term dietary exposure to ethanol.

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225.9 REVERSIBILITY OF DENDRITIC SPINE DENSITY CHANGES IN RAT HIPPOCAMPUS AFTER CHRONIC ETHANOL CONSUMPTION. M.A. King, D.W. Walker and B.E. Hunter. Department of Neuroscience and Center for Alcohol Research, University of Florida, and Veterans Administration Medical Center, Gainesville, FL, 32610.

CNS pathology can often be observed in humans and animals after chronic ethanol exposure. Although some is due to neuronal death, and is therefore virtually permanent, it was not known whether surviving damaged neurons could exercise any regenerative ability. Human CT scan and neuropsychological data suggest that some recovery from cerebral atrophy and intellectual deficits can occur, and data exist to indicate that neurons possess a dynamic morphology. We quantified dendritic spine density in two neuronal populations in a between-groups design to investigate whether some reversibility from cellular neurotoxic sequelae of chronic ethanol consumption is possible.

Male Long-Evans rats were pair-fed 20 weeks with either nutritionally complete ethanol (8.1-9.7% v/v) (CET), or isocalorically substituted sucrose, liquid diets. The 2 x 2 design required sacrifice of 1 alcohol and 1 control group immediately following alcohol removal (A0, S0), and another pair of groups after an additional 20 weeks maintained on ad lib lab chow/water diets (A20, S20). Brains were coded and prepared with a modification of the Scheibels' rapid Golgi technique. Within the dorsal hippocampus, linear dendritic spine density was measured in strata oriens, proximal and distal radiatum, and lacunosum-moleculare for CA1 pyramidal neurons. Dentate granule neuron spine density was sampled in proximal-distal thirds of the molecular layer. In CA1, CET resulted in an initial non-significant reduction in spine density, but A20 group values were significantly higher after 20 weeks "abstinence" than A0, except in stratum lacunosum-moleculare. Granule cells initially showed a significant increase in spine density that also returned toward normal. No S0-S20 differences were found, and spine densities were comparable to those reported by others. Thus, even though differential initial effects were induced, the tendency to return toward normal spine density levels is consistent with the concept of neuronal recovery.

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225.10 SELECTIVE LONG-TERM POTENTIATION IN THE PYRIFORM CORTEX. J. S. Stripling and D. K. Patneau. Department of Psychology, University of Arkansas, Fayetteville, AR 72701.

Electrical stimulation of the olfactory bulb (OB) produces an evoked potential (EP) in the pyriform cortex (PC) characterized by an initial surface-negative wave (period 1) with two components (A1 and B1) which represent, respectively, activation of PC pyramidal cells via the lateral olfactory tract, and feed-forward excitation within the PC. This is followed by a surface-positive wave (period 2) which is temporally associated with inhibition of PC pyramidal cells. Last year we reported that repeated high-frequency stimulation of the OB resulted in both short- and long-term potentiation in the PC which appeared to be selective to period 2, although our electrode placement did not permit a clear assessment of possible changes in B1 as well (Soc. Neurosci. Abstr. 10: 76, 1984). This led us to speculate that increased pyramidal cell excitability might mediate the potentiation of period 2 through recurrent inhibition. Such a mechanism would also result in the potentiation of B1 due to increased feed-forward excitation within the PC. Thus we examined B1 as an operational expression of pyramidal cell excitability.

Male Long-Evans rats were chronically implanted with electrodes in the OB and ipsilateral and contralateral PC. Experimental animals received high-frequency stimulation of the OB in the form of 30 trains delivered at 10-sec intervals; each train consisted of ten 400-microA, 0.2-msec pulses at a frequency of 100 pulses/sec. Control animals received the same number of pulses delivered at a lower frequency (1 pulse/sec). This procedure was repeated three times at 48 hr intervals. Consistent with our previous finding, the experimental treatments produced increasing amounts of short- and long-term potentiation of period 2 in the ipsilateral PC, while the control treatments had negligible effect. This potentiation persisted well beyond its apparent decay in latent form, permitting it to be re-established by low-frequency or paired-pulse stimulation which had little effect in controls. Potentiation of period 2 in the ipsilateral PC was accompanied by the appearance of a component in the contralateral PC of the same polarity and similar latency which could not be elicited in unpotentiated animals.

Neither A1 nor B1 was altered by the experimental treatment, arguing against the possibility that potentiation of period 2 is a consequence of increased pyramidal cell excitability. The polarity of the potentiated component in each PC and the absence of changes in period 1 suggest that investigation of long-term potentiation in the PC should concentrate on inhibitory mechanisms.

225.11 FUNCTIONAL CORRELATES OF SELECTIVE LONG-TERM POTENTIATION IN THE PYRIFORM CORTEX. D. K. Patneau and J. S. Stripling. Department of Psychology, University of Arkansas, Fayetteville, AR 72701.

Recent research from our laboratory has established a selective form of long-term potentiation restricted to late components of the evoked potential (EP) elicited in the pyriform cortex (PC) by olfactory bulb (OB) stimulation (see Stripling and Patneau, this session). The initial wave of the EP, representing both a mono- and di-synaptic EPSP in PC pyramidal cells, remains unchanged, while the second wave, temporally associated with IPSPs in PC pyramidal cells, is altered dramatically by high-frequency trains. The present experiment tested the hypothesis that this potentiation may reflect a functional increase in inhibition within the PC.

In naive animals paired-pulse stimulation produces facilitation of the mono-synaptic EPSP component of the EP (A1) and inhibition of the di-synaptic EPSP component (B1). Facilitation of A1 has been attributed to increased neurotransmitter release due to $Ca^{++}$ accumulation in presynaptic terminals (Bower & Haberly, 1984), while inhibition of B1 is apparently due to recurrent and feedforward inhibition within the PC (Haberly & Bower, 1984). We assessed functional inhibition within the PC by examining the expression of B1 at various interpulse intervals and current intensities in both potentiated and control animals. Paired-pulse depression of A1, a functional change observed only in potentiated animals, was also assessed.

Male Long-Evans rats with chronically implanted electrodes in the OB, ipsilateral PC, and contralateral PC received either high-frequency (100 pulses/sec) or low-frequency (1 pulse/sec) stimulation of the OB at 48 hr intervals. Potentiated animals displayed paired-pulse inhibition of B1 at longer intervals than did controls. Depression of A1 was also observed in potentiated, but not control, animals at brief paired-pulse intervals. This depression of A1 was correlated with a change in the potential recorded in the OB which may reflect increased inhibition of OB mitral cells, resulting in decreased transmission from the OB to the PC. The change in B1 inhibition, however, was not directly related to changes in either the potentials recorded in the OB or the size of A1.

These findings support the conclusion that LTP in the PC represents a selective enhancement of inhibitory mechanisms and suggests that a similar form of potentiation occurs within the OB. The implications of these findings for models of olfactory processing and the functional significance of long-term potentiation will be discussed.

225.12 ON THE ROLE OF POSTSYNAPTIC EXCITABILITY DURING LONG TERM POTENTIATION IN THE RAT SUPERIOR CERVICAL GANGLION. C.A. Briggs and D.A. McAfee. Beckman Res. Inst./City of Hope, Duarte, CA 91010.

Long-term potentiation (LTP) of nicotinic synaptic transmission in the rat superior cervical ganglion is accompanied by an hours-long potentiation of the evoked release of acetylcholine (ACh). While this presynaptic mechanism could account for LTP, our present experiments use exogenous cholinergic agonists to test the idea that postsynaptic mechanisms such as receptor potentiation may also contribute to LTP.

Ganglia were maintained in vitro (21°-24°C) by superfusion with oxygenated Locke's solution containing atropine (2 uM) to block muscarinic transmission. Nicotinic fast-EPSP's elicited by preganglionic stimulation were recorded by intracellular microelectrodes in single postganglionic neurons. Cholinergic agonists were applied by pressure ejection from an extracellular micropipette. Preganglionic tetanic stimulation (20 Hz, 20 sec) induced LTP of nicotinic synaptic transmission. The amplitude of the nicotinic excitatory postsynaptic potential (EPSP) was potentiated by 45%-220% and the amplitude of the depolarizing response to ACh or carbachol was increased by 12%-30% fifteen minutes after the tetani. Both ACh and carbachol are potent muscarinic agonists. Thus, it is possible that these exogenous agonists (0.3-1 mM in the pipette) surmounted the atropine (2 uM) antagonism, allowing the cell to express muscarinic rather than nicotinic potentiation. When dimethylphenylpiperazinium (DMPP; 30-300 uM in the pipette) was used as a selective nicotinic agonist, responses to its application were not potentiated during LTP even though synaptic responses were. LTP was not correlated with measurable changes in membrane potential or resistance in the postsynaptic neuron.

In three other experiments, the sucrose gap technique was used to record from a population of postganglionic neurons in response to preganglionic stimulation, and to superfused DMPP (100 uM, 30 sec). Both responses could be reversibly blocked by nicotinic antagonists. The postganglionic response to nerve stimulation was increased by 72%-103% 15 minutes after preganglionic tetani (20 Hz, 20 sec), but there was no increase in the response to DMPP.

Thus, nicotinic LTP does not appear to be accompanied by increased nicotinic receptor responses in the postsynaptic neuron. However, preganglionic tetani did appear to increase the response to exogenous ACh and carbachol, perhaps by postsynaptic potentiation of muscarinic responses.

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225.13 METHOXYFLURANE DOES NOT BLOCK LONG-TERM POTENTIATION. D.L. Tauck and J.J. Kendig. Department of Anesthesia, Stanford University School of Medicine, Stanford, CA 94305

Long-term potentiation (LTP) is the increase in synaptic efficacy induced by a short burst of presynaptic stimuli. Although the mechanism underlying LTP is unknown, some anesthetics, ketamine and phencyclidine, block LTP while diazepam and sodium thiopental do not (Stringer and Guyenet, 1984, Brain Res. 258:159-164). We were interested in whether or not the volatile, general anesthetic methoxyflurane might interfere with the production of LTP.

Hippocampal slices prepared from male rats (100-150g) were maintained in vitro at 35±0.5°C. The slices were perfused with warmed, oxygenated saline from below and exposed to a flow of humid, warm 95:5 $O_2$:$CO_2$ gas across the top surfaces. A concentric bipolar stimulating electrode was placed in stratum radiatum of field CA1 to activate the Schafer-commissural fibers. An extracellular recording electrode was positioned in stratum pyramidale. Slices equilibrated for 2-1/2 hours after the dissection. By this time the amplitude of the evoked response had stabilized. Only those slices capable of firing at least an 8 mV population spike were studied.

The amplitude of the population spike was measured at several submaximal stimulus intensities to generate an input/output curve (population spike amplitude vs. stimulus intensity). After the input/output curve for a given preparation had been stable for 30 minutes, methoxyflurane was added to the $O_2$:$CO_2$ which aerated the perfusate and flowed over the slices. The concentration of drug in the gas was monitored near the slices. Another input/output curve was generated in the presence of the drug after an evoked response had been stable for 20 minutes. Methoxyflurane has no effect on the input/output curve at 0.1%, a concentration below the anesthetic range in humans (minimum alveolar concentration is 0.16%). Higher concentrations (0.2% and 0.3%) shifted the input/output curve to the right in a dose-dependent manner, depressing both the population spike and the underlying synaptic potential.

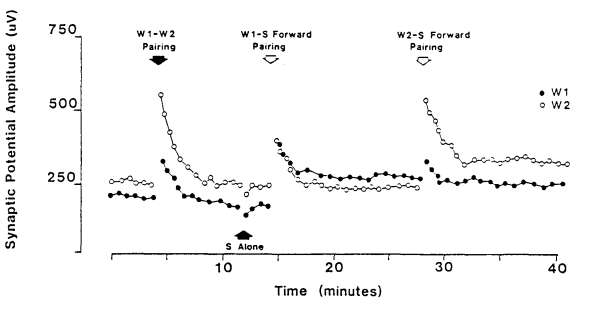
A tetanus (100 Hz), one second in duration, was presented at a stimulation intensity which evoked a 1-3 mV population spike. Fifteen and thirty minutes after a tetanus, the input/output curve was always shifted to the left - indicating the occurence of LTP - whether or not methoxyflurane was present. Therefore, unlike ketamine and phenacyclidine, methoxyflurane does not block LTP in the in vitro hippocampus.

225.14 DIFFERENTIAL INDUCTION OF ASSOCIATIVE LTP. S.R. Kelso and T.H. Brown. Division of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA 91010.

Long-term synaptic potentiation (LTP) in the hippocampus has previously been shown to exhibit associative properties, whereby high-frequency activation of a weak synaptic input will result in a long-lasting enhancement of synaptic efficacy if, and only if, temporally paired with the activation of a separate, stronger synaptic input.

In the present study, three independent stimulating electrodes were placed in region CA1 of the hippocampal slice to stimulate Schaffer collateral/commissural projection fibers. Stimulation intensities were adjusted to elicit a weak synaptic response (200-300uV) with each of two electrodes (W1 and W2) and a much stronger synaptic response (2.5-3.0mV) with the third electrode (S). High-frequency stimulation delivered to W1 and W2 or to S alone (closed arrows), failed to elicit LTP in either W1 or W2. Stimulation trains were then delivered to all three synaptic inputs using differential paradigms (open arrows), in which trains to one weak input began 200 ms before trains to S (forward-paired) and the other weak input began 600 ms after trains to S (backward-paired). With these paradigms, the synaptic response to the forward-paired weak input exhibited associative LTP, while the response to the backward-paired weak input did not.

The occurrence of differential associative LTP demonstrates the potentiality of hippocampal synapses to express the type of associative plastic modifications that could provide a substrate for some of the functions in which the hippocampus is believed to participate. (Supported by NIH grant NS07408, AFOSR Contract F49620, and McKnight Foundation Scholar and Development Awards.)



225.15 **A MUSCARINIC AGONIST (ETHMOZINE) PREVENTS LONG-TERM POTENTIATION IN THE HIPPOCAMPAL SLICE.** J. Krontiris-Litowitz, J. E. Skinner, and L. Birnbaumer*, Sect. Neurophysiol., Depts. Neurol. and Cell Biol., Neurosci. Prog., Baylor College of Medicine, Houston, TX 77030

Long-term potentiation of synaptic efficacy (LTP) is a use-dependent cellular model for learning and memory. In the hippocampus both in vivo and in vitro studies have shown that beta-receptor stimulation is necessary for LTP to occur. Most learning disorders, however, have been associated with loss of cholinergic function. Beta-receptor stimulation of adenylyl cyclase is known to be regulated by cholinergic stimulation of the inhibitory regulatory protein, $N_i$. We therefore hypothesize that cholinergic agonists will inhibit LTP in the hippocampus.

In the CA1 region of the hippocampal slice (350 microns thick) population-spike field-potentials were evoked by low frequency (0.2 Hz) stimulation of the Schaffer collaterals. High frequency stimulation (HFS, 100 Hz for 2 secs) of these same axons was used to elicit LTP. In control experiments, all slices perfused with artificial CSF (CSF) demonstrated LTP (n=5). When lnM of Ethmozine, a muscarinic ligand, was applied to the bath during HFS, 5 of 7 slices failed to manifest LTP. Slices perfused with Ethmozine in the absence of HFS and then washed with CSF prior to the delivery of HFS demonstrated normal LTP (n=5). Thus Ethmozine plus HFS inhibits LTP.

In experiments where each slice served as its own control, HFS was delivered during perfusion with CSF (LTP1), then a second HFS was delivered during perfusion with 1 nM Ethmozine (LTP2). All slices demonstrated LTP1, but LTP2 was blocked in 8 of 15 slices. In the remaining 7 slices, LTP2 was significantly reduced in magnitude ($p<.05$). In 4 slices that had not demonstrated LTP2, a third episode of LTP was attempted, but could not be achieved (LTP3) until 3-h after washout (3 out 4 slices showed LTP3). This long-term effect was confirmed in three additional slices. First LTP was inhibited by pairing HFS with Ethmozine-treatment (1 nM). The Ethmozine was then washed out, and HFS presented once every 15 min. In all 3 slices LTP did not occur until approximately 3-h after washout.

Further studies indicated that the effect of Ethmozine is due to its muscarinic action. In a third series of experiments atropine (100nM) was co-perfused with Ethmozine (lnM) during the HFS. All slices demonstrated LTP (n=7). In a series of receptor binding studies in hippocampal homogenates, Ethmozine displaced [3H]-QNB (1.0 nM) from muscarinic receptors. The mean $IC_{50}$ of Ethmozine was $10^{-5}$M. We conclude that a muscarinic agonist, only in the presence of HFS, inhibits the development of LTP. This use-dependent long-term anti-LTP effect may have an important role in the process of learning and memory.

225.16 INCREASE IN THE MAGNITUDE OF LONG TERM POTENTIATION AFTER ENRICHMENT OF THE SLICED HIPPOCAMPAL TISSUE WITH GM1 MONOSIALOGANGLIOSIDE. A. Wieraszko* and W. Seifert. Max-Planck-Inst. f. biophys. Chemie, Dept. Neurobiology, 3400 Göttingen, W.-Germany.

Brief high frequency stimulation (HFS) of most hippocampal circuits evokes a long lasting increase in their synaptic efficiency. This phenomenon called long term potentiation (LTP) has attracted attention as a potential model for memory (G. Lynch and M. Baudry, Science, 224:1057, 1984). Although the molecular mechanism of LTP is still far from clear, most of the experimental evidence points to the synapse as locus of LTP. Among a variety of synaptic molecules, gangliosides are potential candidates. They are highly and specifically concentrated in nerve ending membranes and are involved in several events taking place at the synapse including direct participation in the neurotransmission (A. Wieraszko and W. Seifert, Neuroscience Letters, 52:123, 1984). The aim of the present investigation was to check whether a change in the ratio of individual endogenous gangliosides can influence the magnitude of LTP in the rat hippocampal slice.

LTP was induced by stimulation of Schaffer collaterals with 3 x 200 Hz, 10 sec intervals. The magnitude of potentiation was calculated taking into account the size of the population spike (record in pyramidal cell layer) before, 2 min and 15 min after HFS. Two approaches were used to modify endogenous gangliosides. First, slices were incubated for 3 hrs with neuraminidase from Vibrio Cholerae (0.13 U/ml). This enzyme liberates sialic acid from polysialogangliosides converting them into GM1. This treatment causes about 1.5-fold decrease in the level of polysialogangliosides and about 3-fold increase in GM1. The enzyme-treated slices showed a higher magnitude of potentiation 2 min (63 %, $n = 44$, $p < 0.001$) and 15 min (44 %, $n = 44$, $p < 0.05$) after HFS.

A similar increase in the size of LTP has been observed when slices were incubated with 71.4 μM GM1 for 3 hrs. It is well known that exogenous GM1 is incorporated into the nerve cell membranes becoming physiologically active. Both, enzyme and GM1 treatments had no influence on the size of the population spike when low frequency stimulation (0.06 Hz) was applied. It is concluded that enrichment of synaptic membranes in GM1 improves the tissue ability to demonstrate plastic changes.

225.17 SYNAPTIC STRUCTURAL ALTERATIONS FOLLOWING NEURONAL STIMULATION T.L. Petit, J.C. LeBoutillier*, and M. Klaiman*. Division of Life Sciences, University of Toronto, Scarborough, Ontario M1C 1A4.

Research from several different areas has indicated that structural changes may occur as a result of synaptic stimulation, suggesting that the synapse might be a critical element in the plastic response of the neuron. The present study was conducted to examine the effects of stimulation on the synapse, using a neurochemical stimulant, in vivo, to avoid the possible confounding effects of electrode damage or in vitro manipulations. Adult male Sprague Dawley rats were injected i.p. with 12 mg/kg kainic acid and observed for varying lengths of time before being sacrificed for neuroanatomical examinations. Kainic acid is known to be a powerful excitatory stimulant, driving cells of the CA3 area into high rates of firing. The axons of the CA3 neurons form the Schaffer Collaterals which project to the stratum radiatum of CA1 pyramidal cells; therefore, this area was examined in detail following kainic acid administration. Animals were anaesthetized and perfused with universal fixative, the hippocampus removed, and the CA area dissected and prepared for electron microscopy. Tissue blocks were stained with either osmium followed by uranyl acetate and lead citrate, or ethanol phosphotungstic acid (EPTA). Photographs were taken and examined with the Bioquant II Image Analysis System. The data indicate a general increase in synaptic parameters following neuronal activation.

This research was supported by a grant from the Natural Sciences and Engineering Council of Canada to T.L.P.

225.18 STRESS IMPAIRS LONG-TERM POTENTIATION IN RODENT HIPPOCAMPUS. Michael R. Foy, Mark E. Stanton, Seymour Levine & Richard F. Thompson. Departments of Psychology and Psychiatry, Stanford University, Stanford, CA 94305

The study of long-term potentiation (LTP) by means of the in vitro hippocampal slice preparation has proven to be both a popular and rewarding technique for investigating neural plasticity. It has been shown with this technique that a number of peripheral hormones and central neurotransmitters, which are associated with stress in intact animals, can modulate LTP when applied directly to the hippocampal slice. In the present study, we demonstrate an impairment of LTP in hippocampal explants taken from rats exposed to inescapable tail-shock.

One week prior to testing, adult male rats were pair-housed in climate controlled facilities on a 12 hr light/dark cycle with food and water available ad lib. One animal in each pair (Stress) was placed in a restraining tube and received tail shocks (1 mA, 1 sec) every minute for 30 min. The other animal in each pair (Control) was taken directly from the home cage and received no restraint or tail shock. In vitro hippocampal slices (400 um) were then prepared immediately from these animals according to standard methods. Extracellular recordings of the population field potential were taken from the CA1 cell body layer in response to stimulation (0.1 msec pulses) of Schaffer collateral afferents, before and after tetanus (100 Hz for 1 sec). There were no differences between the Stress and Control animals in cell excitability pre-tetanus as determined by the stimulus intensity required to produce a population field potential of constant amplitude (1 mV). Within the first minute post-tetanus, post-tetanic potentiation was found in both Stress and Control slices. In contrast, Control animals exhibited LTP (250% of baseline at 30 min), whereas Stress animals exhibited no LTP following tetanus (95% of baseline at 30 min).

These data indicate that stress can significantly modulate neural plasticity within rodent hippocampus. Some of the variability of results seen in previously reported LTP experiments may be accounted for by the behavioral state of the animal. These findings also demonstrate an interface between behavioral manipulations of the intact animal and neurophysiology in vitro in a mammalian model system. Finally, these data raise the possibility that the humoral status of the intact animal may be an important factor modulating in vitro neural plasticity.

Supported by NIMH fellowship 5-32-MH08999 (MRF), NIH fellowship MH-15147 (MES), NIH grant MH-19936 (SL), ONR contract N00014-83-0238 and NSF grant BNS-81-17115 (RFT).

225.19 ROLE OF BRAIN EXTRACELLULAR PROTEINS IN THE MECHANISM OF LONG-TERM POTENTIATION IN RAT BRAIN HIPPOCAMPUS. Victor E. Shashoua and Gary W. Hesse*, Ralph Lowell Laboratories, McLean Hospital, Harvard Medical School, Belmont, MA 02178

In previous studies in this laboratory (Science 212:1148-1151, 1981) long-term potentiation (LTP) of rat brain hippocampal slices was found to enhance the synthesis and release of proteins into the extracellular medium. Pulse-chase experiments (Brain Res. 306:61-66, 1984) showed that the releasable protein pool had an apparent half-life of about 4 h. The major components of this fraction are a class of brain glycoproteins (the ependymins) whose turnover rate increases following the acquisition of new patterns of behavior.

Investigations of the molecular properties of the ependymins indicate that these highly soluble extracellular proteins can rapidly aggregate to form an insoluble fibrous matrix if calcium is removed from the medium by the addition of the calcium chelating agent, EGTA, or by dialysis. This observation led to the proposed hypothesis (Cell. Molec. Neurobiol. 5:183-307, 1985) that the ependymins form an extracellular matrix at local sites where neurophysiological events can cause a transient depletion of $Ca^{2+}$. Such calcium depletion phenomena have been noted following stimulation of rat brain hippocampus in vivo and in vitro (Krnjevic et al., Can. J. Physiol. Pharmacol. 60:1643, 1982). The observed decrease of calcium following LTP is of sufficient magnitude to allow the polymerization of ependymins at the potentiated site and where the matrix formed might then be the framework for structural modification of synapses that have been reported for LTP. We have therefore prepared monoclonal anti-ependymin sera and used them to explore the possibility that ependymin aggregates form at specific sites following LTP. Potentiated, rat brain hippocampal slices were fixed and then stained with the antisera. Immunocytochemical studies at the light microscopic level show the presence of highly fluorescent sites within the stratum radiatum below CA1. Electron microscopic studies of these regions stained by the horseradish peroxidase and the ferretin labeled antibody method show the presence of multiple synaptic endings with extracellular matrices highly staining at local sites. Control unpotentiated slices stained by the same antibody procedure showed no such labeled sites. The results suggest that the ependymins may have an important function in the mechanism of LTP and raise the possibility that these extracellular glycoproteins may have a functional role in modifying neuronal connectivity patterns.

This research was supported by a grant from the NINCDS (09407).

## ACTION POTENTIALS AND ION CHANNELS IV

226.1 DEVELOPMENT OF ION CHANNELS IN DROSOPHILA: LATE EVENTS. L. Salkoff. Dept. Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

An entire picture of developing membrane electrical properties can be observed in the flight muscles (DLM) of Drosophila. This is possible because the DLM can be voltage clamped throughout most of its development, and because mutant analysis aids in the separation of the various currents. The developmental history of membrane electrogenesis begins in the mid-pupal period and extends into the second day of adult life. Of five prominent extrajunctional ion currents which can be observed, only two are fully mature before the adult ecloses from the pupal case. These are the two voltage-activated potassium currents: the fast rapidly inactivating current, $I_A$, and the slowly activating current, $I_K$. A fast transient calcium current ($I_{Ca}$) is the third current to develop and rapidly matures at the time of adult eclosion. Surprisingly, two more ion currents develop in the adult stage: one is a fast, transient $Ca^{++}$-dependent potassium conductance ($I_{Acd}$) that reduces membrane excitability by restricting the amplitude of the spike-like muscle response; another is a late developing inward conductance (Ip) that increases membrane excitability by carrying a noninactivating persistent inward current.

The two late developing currents radically change the voltage responses of the adult DLM membrane. In very young adult cells subjected to a depolarizing stimulus, the membrane responds with action potentials that overshoot 0 mV and show little or no spike adaptation. In older adults the voltage responses no longer overshoot 0 mV and, when subjected to a stimulus of sufficient duration, the membrane undergoes a progressive depolarization that inactivates all transient currents. This results in a depolarized plateau response that can continue for two seconds or more.

Both fast transient $K^+$ currents, $I_A$ and $I_{Acd}$, function in the role of fast spike repolarization. However, the late developing current $I_{Acd}$ can apparently completely supplant $I_A$ in this role. This is seen in the mutants of the Shaker locus which completely eliminate $I_A$ (but not $I_{Acd}$). In young adult fibers before the development of $I_{Acd}$, spikes in mutant fibers are repolarized much more slowly than in wild-type. After the development of $I_{Acd}$, however, the spike repolarization rate in wild-type and mutant fibers is indistinguishable.

(Supported by NSF Grant BNS 8311024 to L. Salkoff.)

226.2 CLONING OF A GENE AFFECTING SODIUM CHANNELS IN DROSOPHILA. K. Loughney* and B. Ganetzky. (SPON: C.-F. Wu) Lab. of Genetics, Univ. of Wisconsin, Madison, WI. 53706

The $para^{ts}$ (paralytic, temperature-sensitive) mutation in Drosphila has been shown to block conduction of nerve action potentials in a temperature-dependent fashion (Wu and Ganetzky, Nature 286:814, 1980). This result together with genetic (Ganetzky, Genetics 108:897, 1984) and pharmacological (Suzuki and Wu, J. Neurogenetics 1:225, 1984) studies suggest that $para^{ts}$ affects sodium channels. To identify and characterize the product of this locus, we have cloned the para gene via transposon tagging. We isolated a new mutant allele, $para^{hd2}$, that arose by insertion of a transposable P element into $para^+$. In situ hybridization to polytene chromosomes confirmed the presence of a P element at the known cytological location of para. Reversion of $para^{hd2}$ back to wild-type was associated with loss of the element from this site.

Using the P element as a molecular "tag" we isolated a DNA fragment from the $para^{hd2}$ strain that mapped to the para region by in situ hybridization. This fragment was used subsequently to isolate from a wild-type library additional overlapping clones of DNA in the para region. The site of the P element insertion as well as an inversion breakpoint (both of which cause a para phenotype) have been localized within a few kilobases of each other on a molecular map of this region. The extent of the para gene is now being further localized by analysis of other chromosome rearrangements that cause para mutations. Attempts to identify the $para^+$ transcript and to isolate a cDNA clone are underway.

(Supported by research grant NS1539 and a Research Career Development Award NS00719 from the NIH.)

226.3 EXPERIMENTAL AUTOIMMUNE PARALYSIS ASSOCIATED WITH VOLTAGE-GATED SODIUM CHANNELS. Y. Rosenthal*, D. Teitelbaum*, T. Brenner* and H. Meiri (SPON: M. Gavish). Dept. of Physiology and Biophysics, Technion Faculty of Medicine, Haifa 31096; Dept. of Chemical Immunology, Weizmann Inst., Rehovot 76100; Dept. of Neurology, Hadassah Medical School, Jerusalem 91010, Israel.

A sodium channel-specific monoclonal antibody (mAb) denoted SC-72-38 modulates channel conductance (Barhanin et al., PNAS, in press). These mAb's bind to a channel epitope at the external surface of the membrane, depress in a dose-dependent reversible manner the compound action potential of mouse sciatic nerve, and label the channels at the node of Ranvier.

Within 7-10 days after injecting hybridoma SC-72-38 into the peritoneal cavity of isogenic mice (CD2F1), walking difficulties developed, followed by complete hind-leg paralysis. The onset and severity of the paralysis were correlated with the development of a myeloma in the peritoneal cavity and the accumulation of ascitic fluid there. This paralytic disease was specific to SC-72-38 and was not found with hybridomas that release mAb's against other sodium channel epitopes or irrelevant sites. Metastases were not found in the mouse nervous system until terminal stages of the disease. Neither hybridoma cells nor blood cells penetrated the peripheral nerve trunk.

Indirect immunofluorescence labeling revealed that hind-leg paralysis was accompanied by mAb infiltration into the sciatic nerve trunk. These mAb's bound to the channels at nodes of Ranvier, thereby depressing impulse conduction of mouse sciatic nerve. Internalization of mAb-channel complexes was then found.

A series of pathological changes was then developed in the Schwann cells that participated in the disease progression. Schwann cell identification was according to their bipolar structure and by the use of two specific markers: 1) a mouse mAb generated against myelin basic protein and 2) a rabbit polyclonal antibody generated against galactocerebroside.

At early stages of the paralysis, Schwann cells at internodal regions respond to mAb infiltration into the nerve by hypertrophy and by increasing their sodium channel density as revealed by immunofluorescence. These channels can then absorb the free mAb's dispersed among the nerve fibers. A partial recovery from the paralysis can then develop as the Schwann cells internalize channel-mAb complexes. However, the segmental demyelination that consequently develops causes severe paralysis. Proliferation of the Schwann cells is then started, and they migrate to the bare axolemma and remyelinate it. These reactive Schwann cells can then contribute channels to the remyelinated axons to support a temporary recovery of impulse conduction. However, continuous nerve invasion by the mAb's that are constantly produced by the proliferating hybridomas depresses the recovery process.

226.4 AGONIST-ANTAGONIST EFFECTS OF BUPRENORPHINE ON ACTION POTENTIALS OF FROG SCIATIC NERVE FIBERS. J. Lee* and G.B. Frank* (Spon. W.F. Dryden), Department of Pharmacology, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7.

Most opioid antagonist drugs are antagonists at low concentrations and agonists at higher concentrations. Thus at low concs they will antagonize the effects of other opioid agonists but at higher concs they will have additive effects. Therefore these opioid antagonist/agonists do not fit the classical mechanism for 'partial antagonists'. This mechanism proposes that 'partial antagonists' produce a smaller effect when they occupy all the drug receptors in a tissue than the effect produced by 'full agonists.' At a high conc the 'partial antagonist' will displace the full agonist from the receptors and produce its maximum effect which is smaller and thus give the appearance of an antagonistic effect. Two further consequences of this mechanism are that at low concs the 'partial antagonist' will produce a response which is additive with the effects of other opioid agonists and that at high concs a complete antagonism will not be produced. Recently, it has been reported that some opioid drugs act like classical 'partial antagonists' (Martin, W.R. Pharmacol Rev., 35: 283,1983). One of these is buprenorphine whose effects we have investigated on the sterospecific opoiod receptor located on frog sciatic nerve A fibers (Hunter, E.G. and Frank, G.B. Can. J. Physiol. Pharmacol., 57: 1171, 1979). These opioid receptors are located on or near the intracellular openings of the sodium channels. The experiments were conducted at room temperature using small bundles of nerve fibres dissected from desheathed nerves. Action potentials were recorded from a sucrose gap apparatus in which the chambers were separated by rubber membranes. The drugs were added to an isotonic KCl solution in one of the end chambers. Thus to reach their site of action on the other side of the sucrose gap, the drugs had to diffuse through the axoplasm of the axons. Action potentials were recorded from 2 to 15 mins for the first hour and every 30 min thereafter. A typical experiment lasted 4 hr. In preliminary experiments it was found that $10^{-6}$ M buprenorphine produced about a 10% reduction in the action potential amplitude and $10^{-4}$ M produced about a 30% reduction. Naloxone, an often used opioid antagonist/agonist, had no effect at $10^{-8}$ M but at $10^{-4}$ M it reduced the action potential by 40%. At $10^{-8}$ M naloxone antagonized the effect of buprenorphine but at $10^{-4}$ M it had an additive effect. Meperindine, a full agonist, at $3 \times 10^{-4}$ M reduced the action potential by about 43%. Buprenorphine ($10^{-6}$ M) had an additive effect with this concs of meperidine but at $10^{-4}$ M it antagonized the effect meperidine. Thus the results obtained so far support the suggestion that buprenorphine acts like a classical 'partial antagonist' on this preparation.

226.5 BATRACHOTOXININ-A ORTHO-AZIDOBENZOATE: A RADIOACTIVE, PHOTOACTIVATABLE PROBE FOR THE BATRACHOTOXIN BINDING SITE OF VOLTAGE-SENSITIVE SODIUM CHANNELS. G.B. Brown. The Neurosciences Program and Dept. of Psychiatry, University of Alabama at Birmingham, Birmingham, AL 35294.

Several neurotoxins acting at the voltage-sensitive sodium channel of excitable cells have provided invaluable tools for the isolation, purification and assessment of the sub-unit composition of this important integral membrane protein. Thus, the tetrodotoxin-binding component has been purified to homogeneity in several laboratories from a variety of sources including rat brain (Hartshorne, R.P. and Catterall, W.A., J. Biol. Chem. 259:1667, 1984), rat muscle (Barchi, R.L., J. Neurochem. 40:1377, 1983) and electroplax of electric eel (Miller, J.A. et al., Biochem. 22:462, 1983). In each case a subunit of $M_r \sim 270$ kDaltons has been identified. In rat brain synaptosomes, α-scorpion toxin also labels two additional components of $M_r \sim 37$ and 39 kDaltons.

Batrachotoxin (BTX) binds to a sodium channel site distinct from that for either tetrodotoxin or α-scorpion toxin. It would therefore be of interest to determine the identity of the BTX-binding subunit. Since solubilization of the sodium channel results in loss of BTX binding (unpublished observation), the experimental approach to this problem must include covalent bonding of a BTX derivative in situ prior to solubilization and purification. To this end, a radioactive and photoactivatable derivative of BTX, [$^3$H]batrachotoxinin-A o-azidobenzoate (BTX-OAB), has been prepared by partial synthesis and found to retain binding affinity and specificity similar to that for BTX itself. The specific activity of this compound is 30 Ci/mmole. Radioligand binding experiments using a vesicular preparation from rat brain indicate that BTX-OAB in the presence of α-scorpion toxin binds to the BTX sodium channel site with a $K_d$=45 nM. Pre-irradiation of BTX-OAB with UV light (254 nm) reduces the extent of specific binding whereas irradiation of the pre-equilibrated toxin/tissue mixture results in irreversible incorporation of approx. 10% of specifically bound label. Initial experiments to identify the site(s) of covalent incorporation reveal that 90% of specifically-bound radioactivity is associated with a low-molecular weight, non-protein component, presumably of lipid origin, suggesting that the BTX binding site lies at the membrane lipid/channel protein interface. Additional studies to characterize the lipid component as well as the protein component(s) which incorporate BTX-OAB are in progress.

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226.6 THE BLOCK OF BATRACHOTOXIN-MODIFIED Na CHANNELS BY TETRODOTOXIN IN NEUROBLASTOMA CELLS. L.-Y.M. Huang, Marine Biomedical Institute, The University of Texas Medical Branch, Galveston, TX 77550.

The block of single batrachotoxin (BTX) modified Na channels by tetrodotoxin (TTX) was studied in neuroblastoma NG108-15 using patch clamp techniques. BTX, a specific activator of Na channels, activated Na channels at hyperpolarized potential (e.g., -90mv) and eliminated both fast and slow inactivation (Huang, L.-Y.M. et al., P.N.A.S., 79:2082, 1982). This specific property of BTX-modified Na channels allowed us to study single Na channels under stationary conditions. Depending on membrane potential, the BTX-modified Na channels were open for tens to hundreds mS which was much longer than those observed in normal Na channels. After the application of TTX at concentrations ranging for 10-100 nM, the channel started to flicker between open and blocked states. The amplitudes of open channels were not affected. Both the histograms of open-state duration and block-state duration could be fitted with single exponentials. The blocking and unblocking rate constants were voltage-dependent. The blocking rate constant increased and the unblocking rate constant decreased when the membrane potential was hyperpolarized. The apparent dissociation constant calculated from rate constants was about 30 nM at 0 mV. This voltage-dependent block of TTX in neuroblastoma cells was qualitatively similar to the voltage-dependent block of saxitoxin observed in BTX-modified Na channels incorporated into planner lipid bilayer (French, R., et al., Biophys. J., 45: 301, 1984).

226.7 **SODIUM CHANNEL BECOMES RESISTANT TO IONIZING RADIATION WHEN ITS CONFORMATION IS MODIFIED BY BATRACHOTOXIN.** **J. E. Freschi and A. Moran*. Physiology Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814.**

**We studied the dose-response relationship between gamma radiation and batrachotoxin (BTX)-stimulated sodium influx in neuroblastoma cells in tissue culture. We also tested the hypothesis that changes in sodium channel conformation may alter the radiosensitivity of the channel.**

**Experiments were done on N18 neuroblastoma cells in 24-well cluster trays. Cells were grown in Delbecco's Modified Eagles Medium supplemented with 10% (vol/vol) fetal bovine serum and 1 mM dibutyryl cyclic AMP for 3 to 4 days. Trays were irradiated in a cobalt-60 facility. Sodium fluxes were assayed using methods modified from those developed by Catterall (Mol. Pharmacol., 20: 356, 1982).**

**We found that gamma radiation inhibited toxin-stimulated $^{22}Na$ uptake at doses beyond a threshold of 200 to 300 Gy. Saturation of the response occurred at doses above 2000 Gy. Over the dose range of 200-2000 Gy there was no increase in nonspecific (leak) influx of $^{22}Na$.**

**To study the effect of channel conformation on radiosensitivity, N18 cells were irradiated under conditions that altered the fraction of sodium channels in the closed, inactivated, or toxin-opened states. Reduction of sodium permeability resulted when the cells were in the closed or inactivated, non-conducting states. However, when the cells were in the toxin-opened, conducting state, gamma radiation had no effect at doses up to 2000 Gy.**

**Our results support earlier electrophysiological studies that showed that high doses of ionizing radiation are required to produce a measurable decrease in sodium permeability. In addition, our data suggest that by changing the sodium channel conformation, BTX appears to alter radiosensitive chemical bonds in the gating or ion-conducting portion of the channel.**

**Supported by AFRRI, DNA, Research Work Unit MJ 00013.**

226.8 USE-DEPENDENT BLOCK OF SODIUM-DEPENDENT ACTION POTENTIALS OF MAMMALIAN CENTRAL NEURONS IN CELL CULTURE BY SODIUM VALPROATE. M.J. McLean and R.L. Macdonald, Dept. of Neurology, Univ. of Michigan, Ann Arbor, MI 48104

Effects of sodium valproate (NaVP) were studied on action potential (AP) firing patterns of mouse spinal cord and cortical neurons in primary dissociated cell culture. Experiments involving intracellular microelectrode recording techniques were conducted in protein-free balanced salt solutions at 37°C. At concentrations equivalent to therapeutic anticonvulsant levels in cerebrospinal fluid (10-120 μM), NaVP limited sustained high frequency repetitive firing (SRF) of sodium-dependent action potentials elicited by long duration (450 msec) depolarizing current pulses in both spinal cord and cortical neurons without abolishing spontaneous activity. In NaVP-containing solution, the maximal rate of rise ($\dot{V}_{max}$), an index of the inward sodium current, declined with the firing of successive APs until firing failed. Steady-state hyperpolarization reduced or prevented the limitation of SRF. Identical paired pulses applied at variable intervals elicited APs at frequencies of 400-500 Hz in control solution, but only 50-200 Hz in NaVP-containing solution. $\dot{V}_{max}$ of a single AP elicited after a conditioning train required less than 50 msec to recover to the velocity of the first AP of the conditioning train in control solution and 90-350 msec in the presence of NaVP. Thus, the limitation of SRF by NaVP was use-, time- and voltage-dependent. The limitation appeared to result from slow recovery of sodium channels from inactivation. The accumulation of channels in the inactive state could then explain the use-dependent reduction of $\dot{V}_{max}$. The occurrence of this in vitro effect at therapeutic concentrations suggests that limitation of SRF is a plausible mechanism, at least in part, for the anticonvulsant action of NaVP.

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226.9 MECHANISM AND SITE OF ACTION OF DELTAMETHRIN IN CRAYFISH AXONS. L.D. Brown* and T. Narahashi. Dept. of Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611

Deltamethrin is one of the most potent synthetic pyrethroid insecticides. It is classified as one of the type II pyrethroids which contain a cyano group at the α position. It has been shown that several type II pyrethroids modify the nerve membrane sodium channels to give rise to a prolonged current in much the same way as type I pyrethroids (without an α cyano group) do (Lund, A.E., and T. Narahashi, Pesticide Biochem. Physiol., 20:203, 1983). However, the degree to which the sodium current was prolonged was much greater with type II pyrethroids than with type I pyrethroids.

This study was aimed at elucidating the mechanism whereby deltamethrin prolonged the sodium current and determining the site of action in the channel. Intracellular microelectrodes were used to record resting and action potentials from crayfish giant axons (19.5°C). Membrane depolarizations of 10 to 40 mV occurred at deltamethrin concentrations between 0.3 μM and 10 μM. With prolonged exposure the action potential was blocked. Prolonged washing did not restore the axon to normal function. Tetrodotoxin (300 nM) antagonized the depolarization caused by deltamethrin indicating that sodium channels are responsible for the action.

In sucrose-gap voltage-clamp experiments with internally perfused crayfish axons (10°C), deltamethrin (10 μM) evoked a slowly decaying tail current following a depolarizing voltage step. The tail current accumulated with repetitive pulsing (1 Hz). The tail current declined to 50% of the initial amplitude within ~ 3 minutes and to baseline within 2 to 30 minutes.

Pretreatment of axons with (-)-cis tetramethrin (100 μM), an inactive type I isomer, has been shown to antagonize the action of the active (+)-isomers of tetramethrin (Lund, A.E., and T. Narahashi, Neurotox., 3:11, 1982). The depolarizing action of 1 μM deltamethrin on intact crayfish axons was not affected by pretreatment with (-)-cis tetramethrin (100 μM). In one sucrose-gap voltage-clamp experiment using an internally perfused axon in which (-)-cis tetramethrin (100 μM) and deltamethrin (10 μM) were perfused simultaneously, there was no antagonism between them. These data are indicative of separate sites of action in the sodium channel for the type I and type II pyrethroids.

Supported by NIH grant NS14143.

226.10 MODULATION OF SODIUM CHANNEL GATING KINETICS BY DELTAMETHRIN. K. Chinn* and T. Narahashi. (SPON: S. Holloway). Dept. of Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611.

Pyrethroids have been shown to alter the kinetics of sodium current in squid and crayfish giant axons. Sodium current is maintained during prolonged depolarization and turns off slowly upon repolarization. The present study examines the effects of the pyrethroid deltamethrin on whole cell and single channel sodium currents of mouse neuroblastoma N1E 115 cells using patch clamp technique. Exposure to deltamethrin (10 μM), slightly reduced (~15%) the whole cell peak inward current evoked by a voltage step from -100 mV to -30 mV and markedly prolonged the current, producing a plateau phase. The decay of the modified current could be expressed by the sum of two exponential functions with time constants of ~5 msec and ~1.3 sec at 11°C, the slow time constant being two orders of magnitude faster than the time constant in modified axons. Tail currents observed on repolarizing the membrane back to -100 mV also decayed much faster (time constants ~2 sec and ~6 sec) than in the axons. The current-voltage curve after drug modification attained a peak at a potential 10 to 20 mV more negative than control, but the reversal potential was not significantly affected. Single sodium channel currents were evoked in excised patches by depolarizing the membrane from -100 mV to -30 mV. After drug application (10 μM), the mean channel open time became very prolonged (~1.7 sec at 11°C) compared to control preparations (~2 msec). Single channel current amplitudes were not affected by the drug. The summed average of single channel current traces obtained during depolarization showed that the drug did not affect the initial rising phase of current but that the current peaked at a later time (5-10 msec in control and ~50 msec after drug exposure). The averaged current declined slowly, attaining almost zero after 4 sec. The channels also remained open on repolarization for periods of up to 30 sec.

In conclusion, the pyrethroid deltamethrin lengthens the open time of individual sodium channels, thereby causing prolonged sodium currents during and after a depolarizing pulse. The modified channels open at potentials more negative than control. Supported by NIH grant NS 14143.

226.11 ACTION OF PUMILIOTOXIN-B (PTX-B) AT THE NEUROMUSCULAR JUNCTION.
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PTX-B, an indolizidine alkaloid obtained from the skin of the neotropical frog Dendrobates pumilio, facilitates and then depresses muscle twitches. The potentiation, unaccompanied by an effect on spontaneous transmitter release, acetylcholinesterase activity or on delayed rectification, was suggested to be related to events involving $Na^+/Ca^{2+}$ exchange (Albuquerque et al., Mol. Pharmacol. 19:411, 1981). The present investigation was carried out to yield further information about the action of PTX-B at the cellular level.

PTX-B, at concentrations as low as 50 nM, produced repetitive activity in the extrajunctional regions of glycerol-shocked muscle fibers of the frog, Rana pipiens. A single transmembrane stimulus evoked either a single action potential with prolonged negative after-potential or a train of action potentials with an average frequency of 60-100 Hz and lasting for >3 sec. In the train, each action potential was super-imposed on prolonged depolarizing after-potential (15-20 mV) of the preceeding action potential. The amplitude and duration of the depolarizing after-potential increased, the train duration decreased in a concentration-dependent manner. At a high concentration of PTX-B, (1.5 μM) the spike amplitude in the train progressively decreased and ultimately was blocked as the magnitude of the after-potential reached its peak (40-60 mV). It seems that the toxin induces repetitive action potentials by elevating and prolonging the depolarizing after-potential.

At low $[Ca^{2+}]_o$ (<300 μM), PTX-B neither induced repetitive action potentials nor prolonged the depolarizing after-potentials. On the other hand, high $[Ca^{2+}]_o$ (10 mM) and the substitution of $Ca^{2+}$ with $Cd^{2+}$ (500 μM) substantially suppressed the repetitive activity but did not abolish the depolarizing after-potential. Further, the toxin failed to induce repetitive activity when the $[Na^+]_o$ was 33% of normal. In addition to these postsynaptic effects, the toxin increased the electrical excitability of the motor nerve terminals and evoked repetitive endplate potentials that are suggestive of a presynaptic action. The data indicate that the toxin may act at presynaptic as well as at postsynaptic sites to evoke repetitive activity in response to a single stimulation. This repetitive activity may be explained by a change in membrane conductance parameters for $Na^+/Ca^{2+}$, which is critical for the generation of repetitive activity in the nerve and muscle. (Supported by USPHS Grant NS-12063.)

226.12 VOLTAGE-SENSITIVE, SPONTANEOUSLY OCCURRING CHLORIDE CHANNELS IN CULTURED SPINAL NEURONES.
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Depolarization of cultured mouse spinal neurones can result in the activation of a Ca-dependent $Cl^-$ current ($I_{Cl(Ca)}$) which may contribute to the regulation of membrane excitability in these cells (Owen, Segal and Barker, 1984). In order to assess the relative sensitivity of the gating of $I_{Cl(Ca)}$ to intracellular $[Ca^{++}]$ and membrane potential, patch-clamp recordings (inside-out configuration) were made from cultured mouse spinal neurones (2-4 weeks). $Cl^-$ channels were isolated using symmetrical Tris-Cl solutions containing 10mM $Na^+$ and 1uM TTX. The pipette solution also contained (mM): 1 $Mg^{++}$, 4 $Ca^{++}$. The bath solution contained 1mM $Mg^{++}$ and $[Ca^{++}]$ was varied between 0.01uM and 0.5uM. $Cl^-$ channels were observed in 20 of the 30 patches studied and at all $[Ca^{++}]$s, although in some cases they were latent for many minutes following patch formation. The dependence of channel activity on bath $[Ca^{++}]$ proved equivocal although channels were clearly active in many patches with free $Ca^{++}$ as low as 10nM. Some patches contained at least two classes of $Cl^-$ channel (based upon amplitude distributions), while in others there was apparently a single population. The following analyses were obtained for such a patch. All voltages refer to the pipette tip potential. Current amplitudes were normally distributed at all potentials between +/- 60mV. The elementary current-voltage relationship was curvi-linear such that the single channel conductance was 49pS at -60mV and 25pS at +60mV. Sub-conductance states were extremely rare in this patch and were not considered in the analysis. Channel openings were both infrequent and brief at positive tip potentials but increased in frequency and tended to occur in bursts at more negative tip potentials. At all potentials studied (-60mV to +60mV) the distribution of channel opentimes could be described by two exponential functions whose time constants (tau) varied with voltage ($tau_1$=ca. 1ms, $tau_2$=5ms at +30mV; $tau_1$=3ms and $tau_2$=23ms at -60mV). Opening probability increased at negative potentials (H=39mV), such that p=0.55 at -60mV. Although this population of $Cl^-$ channels would be expected to be most active at depolarized potentials in the whole cell, their identity and possible involvement in $I_{Cl(Ca)}$ remains to be established.

Owen, D.G., Segal, M. and Barker, J.L. (1984). Nature, 311, 567-570.

226.13 DIVALENT CATION PERMEABILITY OF N-METHYL-D-ASPARTATE CHANNELS.
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The reversal potential of currents evoked by selective agonists acting at kainate, quisqualate and NMDA receptors is similar and close to 0 mV (Mayer & Westbrook 1984 J. Physiol. 354: 29-53); single channel recording also suggests a non selective cationic mechanism since Cs ions permeate through ion channels linked to NMDA receptors (Nowak et al 1984 Nature 307: 462-465). The permeability of amino acid activated ion channels to divalent cations is unknown, but several results suggest differences between NMDA and non-NMDA receptor linked responses: replacement of Na by choline produces a more negative shift of the reversal potential of responses triggered by kainate than of those evoked by NMDA (Mayer & Westbrook 1985 J. Physiol 361: 65-90); Mg ions selectively block ion channels activated by agonists acting at NMDA receptors (Nowak et al 1984; Mayer & Westbrook 1985); in addition hippocampal LTP, which is blocked by intracellular injection of the calcium buffer EGTA (Lynch et al 1983 Nature 305: 719-721), is prevented by selective NMDA receptor antagonists (Collingridge et al 1983 J. Physiol 334: 33-46).

To directly examine the role of divalent cation permeability we measured the reversal potential of responses to the selective agonists NMDA and kainate in mouse spinal neurones under voltage clamp and bathed im media containing either 1 mM Ca + 140 mM Na or 20 mM Ca + 112 mM Na. The reversal potential of NMDA responses varied with the extracellular Ca concentration - 1 mM Ca: + 1.5 ± s.d. 1.0 mV, n = 8; 20 mM Ca: + 13.5 + 2.2 mV, n = 10. The reversal potential of responses to kainic acid was unaltered - 1 mM Ca: + 1.3 + 1.2 mV; 20 mM Ca: + 1.7 + 1.4 mV.

We then considered the possibility that ion channels linked to NMDA receptors behave as multi-ion pores (Hille & Schwarz 1978 J. Gen. Physiol 72: 409-442) and possible interactions between permeant monovalent cations, permeant divalent cations (Ca) and impermeant divalent cations (e.g. Mg). In media containing 100 μM Ca with no added Mg NMDA-activated currents have a positive slope conductance between -80 and 0 mV (Mayer & Westbrook, 1985). However on extending the current voltage relationship over the range -220 to + 30 mV we discovered a region of negative slope conductance between -90 to -150 mV; this may be a consequence of contamination by residual Mg estimated at 5 μM. At potentials negative to -150 mV NMDA current voltage plots show an increase in slope suggesting that Mg ions may permeate if the electric field is made large enough; Ca ions may also knock Mg ions past the blocking site since the NMDA current voltage plot at negative potentials became more linear in 5 mM Ca.

226.14 GAP JUNCTIONS: PERMEABILITY TO TEA AND CONDUCTANCE ARE PROPORTIONAL, SUGGESTING ALL-OR-NONE GATING.
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The permeability ($P_j$) of gap junctions to tetraethylammonium ions (TEA) was measured in pairs of blastomeres isolated from embryos of Rana pipiens. Junctional conductance ($g_j$) was quickly and reversibly modulated in a double voltage clamp procedure, and the changes in $P_j$ to TEA were evaluated. Intracellular activity of TEA was measured with ion-selective electrodes filled with Corning liquid ion exchange resin (code # 477317) which is highly selective for quaternary ammonium ions. $P_j$ to TEA was proportional to g among cell pairs that were maintained in the maximally coupled state (transjunctional voltage held at zero). $P_j$ for a 1 uS conductance was 6.5 E (-10) $cm^3$sec. When $g_j$ was reduced by application of a transjunctional voltage, the rate of transfer depended upon the polarity of the applied potential. Although equal $g_j$ values were obtained with $V_j$'s of equal magnitude but opposite polarity, moderate hyperpolarization of the TEA-injected cell greatly reduced the rate of transfer relative to similar hyperpolarization of the recipient cell. When corrected for the additional driving force that transjunctional voltage imposed on the TEA ions, $P_j$ was still linearly related to $g_j$. TEA ions are about 8A in diameter. Presumably $g_j$ is largely determined by permeability to K+ ions which are 6A in diameter. Thus the proportionality of TEA permeability to $g_j$ suggests that channel closure induced by voltage is all-or-none.

227.1 POTASSIUM CURRENTS IN FROG CARDIAC MYOCTYES. EFFECTS OF ACETYLCHOLINE, BARIUM AND POTASSIUM. M. A. Simmons and H. C. Hartzell*. Anatomy Dept., Emory University, Atlanta, Ga. 30322

Previous studies have revealed the presence of three K currents in the heart: a time-independent inwardly rectifying current ($I_{K1}$), a delayed outward current ($I_K$) and an ACh-activated current ($I_{ACh}$). Some disagreement exists regarding the exact number of components of these currents and whether or not each current is independent. This confusion may be partly due to the syncytial nature of cardiac tissue complicating the analysis of voltage-clamp studies using multicellular preparations. We have used the whole cell recording configuration of the patch-clamp technique to record potassium currents in enzymatically dissociated single cells from frog atrium. Na currents were blocked by TTX and Ca currents with Mn. In response to 100 msec voltage steps, a quasi-instantaneous current was observed, $I_{K1}$. The instantaneous current-voltage relation for $I_{K1}$ exhibited inward-going rectification with a negative slope between -60 and -20 mV. Ba (5-500 $\mu$M) blocked this current in a concentration-dependent manner (85% inhibition at 50 $\mu$M). Increasing $[K]_o$ greatly increased this current negative to $E_{rev}$. With longer depolarizations positive to -30 mV, a time-dependent outward current was observed, $I_K$. This current has been fully characterized according to its activation, time constants and ion transfer function. $I_K$ was maximally activated at +20 mV. The time constants of activation of $I_K$ were rather long, ranging from 1 sec at +30 mV to 4 sec at -30 mV. The fully activated current-voltage relation was linear. Ba (250 $\mu$M) did not affect $I_K$. The current elicited in response to iontophoretic ACh at different membrane potentials exhibited inward rectification. In contrast to $I_{K1}$, however, increasing $[K]_o$ decreased the ACh conductance negative to $E_{rev}$. Additionally, $I_{ACh}$ was less sensitive to Ba blockade than $I_{K1}$ (45% inhibition at 50 $\mu$M). ACh did not affect $I_K$. These results indicate that ACh does not act on the $I_K$ channel and that the K channel activated by ACh differs in its properties from the $I_{K1}$ channel. Hence, it is concluded that ACh opens a unique K channel in frog atrium.

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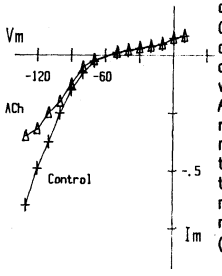
227.2 ACETYLCHOLINE ALTERS THE CURRENT THROUGH THE INWARD RECTIFIER CHANNEL OF THYMUS CELLS IN CULTURE. F. Moody-Corbett and P. Brehm, Dept. Physiology, Tufts University School of Medicine, Boston, MA

Cells of the thymus can express both nicotinic and muscarinic acetylcholine (ACh) receptors (Rossi and Trenkner, Neurosci. Abs., 725, 1984). In order to understand the functional properties of these receptors we have been using the method of whole cell voltage clamp to examine the effects of ACh on thymus cells grown in culture.

The thymus was removed from 8-10 week old rats and individual cells dispersed by gentle shearing. The cells were plated on tissue culture dishes and fed every 3-4 days with Dulbecco's Modified Eagle Medium buffered with sodium bicarbonate, supplemented with 10% fetal calf serum and penicillin-streptomycin. Whole cell voltage clamp recordings were made using conventional patch clamp techniques. The electrode contained (mM) 140 KCl, 1 BAPTA, 10 HEPES ( pH 7.3) and the external recording solution contained (mM) 140 NaCl, 5 KCl, 1 $CaCl_2$, 1.6 $MgCl_2$, 10 HEPES (pH 7.3) with $10^{-6}$g/ml TTX.

When cells were held at -60 mV and stepped to different membrane potentials, the resultant instantaneous currents were highly non-linear at potentials more negative than -70 mV (see Fig.). The current-voltage relations obtained during application of 10 $\mu$M ACh indicate a reduction in the amount of inward rectification observed at potentials more negative than -90 mV (see Fig.). In contrast to this effect on hyperpolarizing potential jumps, no effect of ACh was observed on currents recorded in response to depolarizations to potentials more positive than -60 mV. The effects of ACh were also observed as a decrease in net inward current provided that the cells were held at potentials sufficient to activate inward rectification. Similar results were obtained with 10 $\mu$M muscarine. Addition of 5 mM CsCl to the recording solution linearized the current-voltage relations suggesting that potassium current mediates the inward rectification. In cells in which the rectification was blocked by Cs, no effects of ACh on holding current or the current-voltage relations was observed. These effects of ACh and muscarine suggest that cholinergic agonists cause a transient decrease in potassium current flowing through the inward rectifier channel. This modulation may play an important role in the immunological response of thymus cells.




(Supported by the Myasthenia Gravis Foundation)

227.3 PROPERTIES OF THE INWARDLY RECTIFYING POTASSIUM CHANNEL IN ACUTELY DISSOCIATED HIPPOCAMPAL PYRAMIDAL CELLS. J. R. Huguenard and B. E. Alger, Univ. of MD. Sch. Med., Baltimore, MD 21201.

Modulation of K-dependent conductances is an important mechanism of neuronal excitability control. We have begun to study modulation of K channels in neurons using the acutely dissociated hippocampal cell preparation (1). There is at least one, and perhaps two, large K-dependent channels which can be recorded from hippocampal neurons. The channel conductances range from 100-250 pS, depending on the recording conditions. One channel shows inward rectification (2,3) while the other is similar to the large Ca-dependent K channel which has been characterized in several different cell types (2).

With an on-cell patch configuration and 150 mM K and 0 Ca in the patch electrode we predominantly record large unitary inward currents. The single channel conductance shows inward rectification. In the region of -100 to -120 mV patch potential (assuming -50 mV resting potential) the conductance is about 200 pS, whereas at the resting potential it is approximately 100 pS. The channel is markedly voltage-dependent; hyperpolarization decreases the probability of channel opening. Single channel currents recorded on-cell cannot be reversed with depolarization. If, however, the electrode is withdrawn to obtain an inside-out patch in 150 mM K, a large channel with a linear conductance of about 250 pS is observed. The single channel currents in this case are readily reversible near 0 mV. On the other hand, when the patch electrode contains 2 mM Ca, depolarizations near 100 mV can activate a large outward channel with a conductance of about 100 pS.

We examined some properties of the inward rectifier channel to investigate its relationship to the macroscopic inwardly rectifying K conductance (`Q' current) in hippocampal neurons (3). With the inside-out patch configuration we find that this channel activity can be reversibly abolished by a low calcium (Ca)-containing solution ($\leq 10^{-8}$ M) or by ATP (0.5 mM in $10^{-7}$ M Ca). These properties typify large Ca-dependent K channels in other cell types. Interestingly, carbachol, 10-50 uM, can reversibly inhibit both large inward and outward channels in an on-cell patch.

This evidence confirms previous observations of a large inwardly rectifying K channel in hippocampal neurons. The channel has features which are unexpected based on the properties of the inwardly rectifying `Q' current; namely, inhibition by: a) low Ca, b) large hyperpolarizations, and c) carbachol. The observation that both types of large K channel can be blocked by carbachol suggests that some of the excitatory effects of cholinergic neurotransmission are due to this inhibition.

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2. Numann, R. and R.K.S. Wong, Biophys. J., 47:475a, 1985.
3. Sullivan, J.M. and S.A. Cohen, Biophys. J., 47:385a, 1985.

227.4 THREE OUTWARD IONIC CURRENT COMPONENTS OF NEURONS FROM CAT SENSORIMOTOR CORTEX. W. Spain*, P.C. Schwindt, M.C. Chubb and W.E. Crill, Dept. of Physiology & Biophysics, Univ. of Washington Sch. of Med., Seattle, WA 98195.

Outward ionic currents of large layer V neurons from cat sensorimotor cortex were investigated in an in vitro brain slice using a single microelectrode voltage clamp (SEVC). Sodium currents were abolished by TTX. During voltage steps from a holding potential at resting potential (RP: -60 to -80mV) three distinct outward current components could be distinguished.

A current qualitatively similar to $I_A$ of Connor & Stevens (J.Physiol. 213:21-30, 1971) was first seen at -55mV. Inactivation commenced at -65mV and was complete at about -45mV. Thus, $I_A$ is only partially inactivated in the subthreshold voltage range in these cells. Peak $I_A$ occurred within 2-5 ms in different cells. The time constant of $I_A$ inactivation showed little voltage dependence in a given cell and varied from 2.5 to 7 ms among cells. Because of its rapid onset and inactivation properties, $I_A$ may play a role in spike repolarization in addition to influencing membrane potential during repetitive firing.

A second transient outward current ($I_{KT}$) first appeared at about -50mV. Inactivation of $I_{KT}$ commenced at about -60mV and became complete at about -40mV. $I_{KT}$ also had a rapid initial rise, but inactivated much more slowly than $I_A$ (time constant: 350 ms at -40mV). Qualitatively, this current resembles $I_K$ of molluscan neurons. One likely function of $I_{KT}$ is spike repolarization.

A steady, non-inactivating current ($I_{KS}$) became dominant at potentials positive to -40 mV, but was mixed with $I_{KT}$ at more negative potentials. $I_{KS}$ could be activated when $I_{KT}$ was completely inactivated. Because of the great conductance increase associated with $I_{KS}$ activation, it could be studied only over a limited voltage range with the SEVC. $I_{KS}$ appeared to develop with 2 time constants: a rapid onset was complete in about 60 ms, whereas a subsequent slow phase did not reach a steady value during 1 sec voltage steps. $I_{KS}$ deactivated with a time constant of 180 ms or longer at RP. $I_{KS}$ appears to be composed of at least 2 pharmacologically separable subcomponents, one of which is sensitive to muscarine (Chubb et al, Neurosci. Absts. 10:1181). An $I_{KS}$ -like current was observed after somatic outward currents were blocked by somatic injection of $TEA^+$ or $Cs^+$, and this current was largely eliminated by substitution of $Co^{++}$ for $Ca^{++}$ in the perfusate. These observations suggest that one component of $I_{KS}$ is largely dendritic and, furthermore, is $Ca^{++}$-mediated. $I_{KS}$ activation appears to be responsible for the long-lasting afterhyperpolarization seen in these cells following repetitive firing (Schwindt et al, Neurosci. Absts. 10:871). Supported by NIH grants NS16792 and GM07266.

227.5 VOLTAGE CLAMP ANALYSIS OF RAPIDLY ACTIVATING POTASSIUM CURRENTS IN BULLFROG SYMPATHETIC GANGLION CELLS. B. Lancaster,† P. Pennefather & P. R. Adams. Dept. of Neurobiology, SUNY Stony Brook, NY 11794. †Faculty of Pharmacy, University of Toronto, Ont. M5S 1A1.

We have examined the degree to which the fast Ca-activated K current ($I_C$) and the delayed rectifier ($I_{DR}$) can be evoked by short depolarizing commands in bullfrog ganglion cells. Two microelectrodes were used to voltage clamp the cells as described previously (Adams, Brown & Constanti, J. Physiol. 330: 537).

Depolarizing voltage jumps of 2-3 msec duration from holding potentials of -40 mV evoke an inward Na current followed by a large outward current. The I/V relation at the end of the pulse shows a negative conductance region between +20 and +70 mV. This is attributed to a decrease in $I_C$ as Ca current declines at more positive potentials (>+20 mV) followed by an increase in $I_{DR}$.

The contribution of $I_{DR}$ to total outward pulse current is observed more clearly in the outward tail currents after repolarization. $I_{DR}$ contributes a slow component (decay $\tau$ =10-20 msec at -40 mV, showing an e-fold change per 22 mV) to the total outward tail which appears after voltage jumps to >+40 mV. After commands to <+40 mV, tail currents are well described by a single exponential with faster time constant (decay $\tau$ =2-4 msec at -40 mV, showing an e-fold change per 40 mV). The fast tails correspond to $I_C$ since they are abolished by Cd or 0 Ca. These results are consistent with a major role for $I_C$ in spike repolarization.

During very positive commands, $I_C$ contribution to total outward current should be small as $E_{Ca}$ is approached. However, $I_C$ tails, after a peak at +20 mV, decline to a non-zero limiting value after steps to >+70 mV. These residual outward tail currents may be generated by Ca tail currents.

A complication in examination of tail currents is K accumulation resulting from large K efflux during the pulse. Assuming 140 mM $[K]_i$, $E_K$ is at -101 mV in 2.5 mM $[K]_o$. The reversal potential for $I_C$ tails after a 2 msec command to 0 mV (typically evoking 50 nA of outward current) is -55 mV, indicating that $[K]_o$ is 16 mM. Longer commands cause even greater K accumulation, generating a sag in the outward current. Attempts to alleviate K accumulation with high $[K]_o$ Ringer are further complicated by effects of raised $[K]_o$ to increase $I_{DR}$. Indeed, in the presence of 40 mM $[K]_o$ a negative slope conductance region is no longer observed between +20 and +70 mV even though analysis of tail currents indicate that $I_C$ is decreasing in this range.

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227.6 A MONOCLONAL ANTIBODY WHICH SELECTIVELY BLOCKS TWO TYPES OF IONIC CHANNELS IN MAMMALIAN NEURONS : A PATCH-CLAMP STUDY AND IMMUNOFLUORESCENT VISUALIZATION. Irene Zeitoun*, Hamutal Meiri, Carla Distasi* and Michel Simonneau*. Dept. Physiol., Rappaport Family Institute for Research in the Medical Sciences, Technion-Israel Institute of Technology, Haifa 31096, Israël; *Laboratoire de Neurobiologie Cellulaire & Moléculaire, CNRS, 91190 Gif sur Yvette, France.

Monoclonal antibodies (MAb's) were generated against the eel electroplax membrane fragments (EMF). The selection of suitable antibodies, which could modify the channel activity, was made according to (i) a Toxin-Modulated Solid-Phase Radio-Immuno-Assay (TM-SP-RIA), using as antigen the EMF and rat brain synaptosomes (RMV), with the same density of $Na^+$ channels, to test cross reactivity,(ii) an immunofluorescent staining technique (Meiri H. et al., Brain Res., 310, 168-173, 1984). The binding of one clone, SC-79-17 ($IgG_1$), to EMF and RMV was decreased by veratridine, but scorpion toxin was ineffective. TTX inhibited this MAB binding only to RMV. This clone stained nodes of Ranvier of rat and mouse sciatic nerve. It increased and prolonged the compound a.p. of rat optic and sciatic nerve, then partially blocked it. TTX which blocks $^{22}Na$-flux was more effective in the presence of SC-79-17, suggesting a close association between these two sites (TTX and antibody sites). Patch-clamp studies revealed that SC-79-17 blocks partially whole-cell $Na^+$ and $K^+$ currents, in embryonal teratocarcinoma that had differentiated into neurons (Eddé et al, J. Physiol, 346, 82P, 1984) and in mouse neonate dorsal root ganglion cells. The whole-cell $K^+$ current blocked by the MAb was a $Ca^{++}$ activated $K^+$ current. $Ca^{++}$ currents were not affected. Application of the MAb on outside-out patches provided evidence that only the large conductance (200 pS in symmetrical $K^+$) $Ca^{++}$ activated $K^+$ channels were blocked by SC-79-17.

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227.7 A $Ca^{++}$-DEPENDENT $K^+$ CURRENT IS INCREASED IN THE PARAMECIUM MUTANT, teaA. T.M. Hennessey, Y. Saimi, and C. Kung (SPON: M.D. Bownds). Laboratory of Molecular Biology and Department of Genetics, University of Wisconsin, Madison, Wisconsin 53706.

One advantage of using Paramecium as a model system for studying ion channel functions in excitable membranes is that their membrane ion channels can be altered by mutation. Paramecium is a eukaryotic, unicellular ciliate which swims by the coordinated beating of the thousands of cilia which cover its body. Depolarizing stimuli trigger the opening of voltage dependent $Ca^{++}$ channels to produce $Ca^{++}$ action potentials and consequent ciliary reversals. Ciliary reversals cause the cell to swim backwards. Since the swimming behavior is dependent upon proper ion channel functions, behavioral mutants can be generated which have electrophysiological defects. The behavioral mutant teaA does not dramatically alter its swimming behavior in depolarizing solutions (such as $TEA^+$ and $Na^+$) whereas wild type show ciliary reversals and backward swimming.

Voltage clamp analysis has shown that the teaA mutant has a larger $Ca^{++}$-dependent $K^+$ current than wild type (in a $Ca^{++}$-$K^+$ solution). There are no other changes in ionic conductances in teaA. The large $Ca^{++}$-dependent $K^+$ current of teaA can be eliminated by: 1) removing the inward $Ca^{++}$ current. This was done by constructing the pawnB-teaA double mutant. The pawnB virtually lacks the inward $Ca^{++}$ current but is not allelic to teaA, 2) blocking the $Ca^{++}$-dependent $K^+$ current with internal $Cs^+$ (this current is not efficiently blocked by external $TEA^+$), and 3) chelating internal $Ca^{++}$ with injected EGTA. Tail current analysis showed that the $Ca^{++}$-dependent $K^+$ current was not only larger in teaA but faster activating as well. The activation of this current is so fast in teaA that it short-circuits the action potential and inhibits depolarization induced ciliary reversals in the free swimming cell.

Supported by NSF grant BNS-82-16149 and NIH grant GM 22714 to C. Kung.

227.8 GENETIC ALTERATIONS OF SINGLE-CHANNEL CURRENTS IN DISSOCIATED CNS NEURONS OF DROSOPHILA. Y.-A. Sun and C.-F. Wu. Dept. of Biology, Univ. of Iowa. Iowa City, IA 52242.

Ionic channels are integral membrane proteins and may be subject to genetic manipulation. Mutations of Hk and eag genes in Drosophila have been shown to increase nerve excitability. Using dissociated CNS neurons from Drosophila larvae, we analyzed the effects of these mutations at the single-channel level. Different types of $K^+$ currents were recorded from cell-attached membrane patches on the soma of type III neurons and neuroblasts (Wu et al., J. Neurosci. 3:1888, 1983). The cells were bathed in Drosophila physiological saline, with the addition of 3 μM TTX, 5mM $CoCl_2$ and 1 mM $CdCl_2$ to block $Na^+$ and $Ca^{++}$ channels. The patch electrodes were filled with the same solution.

Three distinct classes of single-channel $K^+$ currents can be readily recorded from these cells, namely, delayed rectification, inward rectification and $Ca^{++}$- dependent $K^+$ currents. Previous voltage-clamp measurements demonstrated that the amplitude of the delayed rectification current is reduced in $eag^1$ larval muscles (Wu et al., Science 220:1076, 1983). Single-channel recordings from $eag^1$ cultured nerve cells revealed that a decrease in channel conductance (to about 60% of the normal value, 11 pS) rather than in channel density is responsible for the reduction of the macroscopic current. This also raised the possibility that the eag gene may code for a structural component of the delayed rectification channel.

A population of CNS neurons in $Hk^1$ flies have been reported to display abnormal pace-maker activities not found in normal neurons (Ikeda and Kaplan, PNAS 66:765, 1970). In cultured $Hk^1$ nerve cells inward currents of an unusually high unit conductance (about 40 pS, as compared to 16 pS of normal inward rectification channels) were activated by membrane hyperpolarization. Preliminary analysis indicates that this may reflect a novel conductance state of the inward rectification channel. Since the inward rectification current reverses at a potential more positive (> 20 mV) than the resting potential, activation of these high conductance channels may greatly increase neuronal excitability.

Supported by NIH grants NS 00675 and NS 18500 and a grant from the Searle Scholars Program.

227.9 CLONING OF A GENE AFFECTING POTASSIUM CHANNELS IN DROSOPHILA. R. Drysdale* and B. Ganetzky. Lab. of Genetics, Univ. of Wisconsin, Madison WI 53706

The eag mutation in Drosophila causes spontaneous repetitive firing of action potentials in motor axons and abnormal release of transmitter at the larval neuromuscular junction (Ganetzky and Wu, J. Neurogenet. 1:17, 1983). The results from voltage clamp studies of larval body wall muscles (Wu et al., Science 220:1076, 1983) as well as single channel recordings from dissociated neurons (Sun and Wu, Abstr. Soc. Neurosci. In Press) indicate that eag causes a reduction in a delayed rectification potassium current ($I_K$).

To learn more about the product of this gene and its role in membrane excitability we have isolated clones of the eag locus. The cloning strategy took advantage of a chromosome inversion broken at one end in the eag locus and at the other in a previously cloned region of the chromosome. A junction fragment of DNA, which includes a portion of the eag locus, was isolated by screening a genomic library of the inversion using available probes from the cloned segment. The eag portion of the junction fragment was then used to screen a wild-type library to isolate additional overlapping clones in the eag region. We are now defining the extent of the eag gene within this cloned region by analyzing at the molecular level chromosome rearrangements and transposon insertions that cause mutant eag phenotypes.

(Supported by research grant NS1539 and a Research Career Develoment Award NS00719 from the NIH).

227.10 CALCIUM AND CYCLIC AMP-MEDIATED REGULATION OF Ca AND K-CURRENTS IN HERMISSENDA GIANT NEURONS. Juan Acosta-Urquidi. MBL, Woods Hole, MA 02543 and Friday Harbor Laboratory, Friday Harbor, WA 98250.

Previous work employing voltage-clamp techniques has addressed the role of 2nd messenger-mediated modulation of ICa and distinct K-currents in giant identifiable pedal and pleural neurons in the Hermissenda CNS. Intrasomatic injection of two distinct species of Ca-calmodulin (CaM) dependent kinases, phosphorylase kinase and TACK (J. Biol. Chem. 258:12632, 1983), produced complex modulation of K-currents and ICa (Soc. Neurosci. Abstr. 9:501, 1983; 10:1129, 1984). Further studies showed CaM-KII (labelled TACK) predominantly enhanced ICa and IBa (ave. 31.2%, N=18 cells, P<.001), but in some cells, increases and decreases of ICa were obtained. CaM-KII also reduced both delayed rectifier, IK(v) (30.4%, N=7, P<.005), and IA (27.8%, N=6, P<.01). Increased rate of IK(v) inactivation was also observed in some cases. Effects on IK(Ca) tracked the effects on ICa, but further work is needed to detect CaM-KII direct effects on IK(Ca). In cells LP1-3, 5-HT (1-10 uM) predominantly enhanced ICa and IK(Ca) and consistently reduced leakage conductance (Soc. Neurosci. Abstr. 10:145, 1984). 8-BTcAMP (1 mM) also reduced leakage and increased ICa. Leakage is reduced by Cd (1 mM) and 4-AP (5 mM), but not EGTA injections. 5-HT effects were re-examined, including pleural cells. In Ba-ASW, to remove IK(Ca) contamination, 5-HT (10 uM) predominantly reduced IBa (40%, 7/9 cells) and consistently reduced leakage conductance (-50 to -120 mv, from Vh-40 mv). Forskolin (FORSK, 25-50 uM), an activator of adenylate cyclase, reduced leakage (40%) in Ca and Ba TEA ASW plus added Cd. FORSK reduced IBa (45%, 7/8 cells) and reduced IA and increased its decay rate, as reported (J. Neurosci. 4:2772, 1984). FORSK also reduced IK(v) (40%, 7/8 cells), increased IK(v) decay rate, increased IK(v) twin pulse inactivation (P1/P2), and delayed recovery of inactivation following preconditioning hyperpolarization. The wide range of effects of Ca-CaM and cAMP-mediated effects on diverse ionic currents poses new questions regarding the substrate specificity for the phosphorylation-mediated regulation of channel activity.

I thank D. L. Alkon, Lab. of Biophysics, NIH, MBL, for the use of facilities.

227.11 $I_{K-Ca}$ BUT NOT $I_A$ IS REDUCED DURING IN VITRO CONDITIONING OF HERMISSENDA TYPE B CELLS. (Spon: R.Cholewiak). L.Grover* & J.Farley, Prog. in Neurosci. & Behavior, Princeton Univ., Princeton, NJ 08544.

Pairing-specific reductions in two $K^+$ currents ($I_A$, Alkon et al., Science, 1982; and $I_{K-Ca}$, Farley & Alkon, Farley et al., Soc. Neurosci. Abstr. 1983,1984) have previously been described for Type B photoreceptors on retention days following learning. Cumulative depolarization of B cells has been proposed as one acquisition mechanism leading to the long-term reduction of $K^+$ currents. We have asked whether the reductions in $I_A$ and $I_{K-Ca}$ observed during retention are also produced during acquisition of learning.

Under current clamp, axotomized B cells were exposed to an in vitro simulation of the associative conditioning procedure. Cells were exposed to either 5 pairings (ITI=1.0 min) of light (110 μW; 15 sec) followed immediately by 15 sec of depolarizing current stimulation (Vm= ∿-20 mV), or five unpaired presentations of these same stimuli, with cells receiving both treatments in a counterbalanced order. 10 of 13 cells exhibited a pairing-specific cumulative depolarization ($\bar{x}$ ± s.d.; paired: 6.1 ± 2.2 mV; unpaired: 4.4 ± 1.8 mV; p <.05), when measured 1.0 min following training. B cells were also exposed to the in vitro conditioning protocols under voltage-clamp control ($V_h$=-60 mV) and comparisons of $I_A$ and $I_{K-Ca}$ were made before and after conditioning. $I_A$, measured as the peak outward current during 500 msec command steps to either -10 or 0 mV, showed no consistent reduction by either paired or unpaired training conditions (see Table 1, below). In contrast, $I_{K-Ca}$ (measured as the steady-state outward current in a 5 mM 4-AP; 100 mM TEA ASW; at both -10 and 0 mV) was reduced in a pairing-specific manner (Table 1). Thus, $I_{K-Ca}$, rather than $I_A$, undergoes persistent, pairing-specific reductions during acquisition, which are independent of any cumulative voltage-dependent inactivation effects.

Table 1

| | $I_A$ (mean ± s.d.; nA) | | | | $I_{K-Ca}$ (mean ± s.d.; nA) | | | |
|---|---|---|---|---|---|---|---|---|
| | -10 mV | | 0 mV | | -10 mV | | 0 mV | |
| | pre | post+ | pre | post+ | pre | post+ | pre | post+ |
| paired (n=8) | 20.0 ±4.6 | 20.1 ±5.3 | 37.7 ±12.8 | 35.4 ±13.6 | 5.2 ±1.7 (n=6) | 4.1 ±1.2 | 8.2 3.0 | 6.8 1.8 |
| unpaired (n=6) | 25.0 ±12.5 | 25.8 ±10.7 | 45.4 ±19.8 | 42.3 ±18.3 | 4.8 ±0.9 (n=6) | 4.4 ±1.2 | 7.0 ±2.0 | 6.5 ±1.5 |

+1 min post

Supported by NSF BNS 8316707 to JF

227.12 PROTEIN KINASE C REDUCES $K^+$ CURRENTS AND ENHANCES A $CA^{2+}$ CURRENT IN HERMISSENDA TYPE B CELLS. (Spon: J.Gaddy) D.Fisher*, S.Auerbach, J.Farley. Prog. in Neurosci., Princeton Univ., Princeton, NJ 08544.

Persistent associative-training-produced changes of two $K^+$ currents ($I_A$[1]; $I_{K-Ca}$[2,3]) and a calcium current ($I_{Ca}$[3,4]) have previously been demonstrated in Hermissenda Type B photoreceptors. The same conductance changes are also effected by serotonin (5-$HT^5$), an endogenous neuromodulator released during associative training[6]. It has been suggested that these changes arise from the phosphorylation of ionic channels. But the identity of the phosphorylation pathway(s) has not as yet been determined. We[7] have previously reported that the tumour promoting phorbol esters, which are specific activators of protein kinase C, produce the same conductance changes resulting from associative training and 5-HT. We now report that intracellular injections of the enzyme produces the same effects.

Iontophoretic injections of PKC, prepared from mammalian brain[8], enhanced the magnitude of the B cells' steady-state light response (before: 19.6 ± .9 mV; after: 23.5 ± .07 mV; p < .05) and input resistance (39.3 ± 1.9 MΩ vs. 57.6 ± 2.1 MΩ ; p<.05) in 13 of 13 cells tested. Heat-inactivated PKC (n=10), buffer (n=5), or material from a non-PKC peak eluting from the DEAE cellulose column (n=3) were all without effect. Under voltage-clamp control ($V_h$=-60 mV), PKC injections reduced $I_A$ (peak outward current at -10 mV; before: 31.7 ± 1.8 nA, after: 10.2 ± 0.8 nA) in 10 of 10 cells, reduced $I_{K-Ca}$ (measured in 10mM 4-AP and 100 mM TEA ASW at -10 mV: 16.3 ± 1.2 nA vs. 8.7 ± 0.9 nA) in 8 of 8 cells, and enhanced $I_{Ca}$ in 7 of 7 cells [measured in 4-AP, TEA, and 300 mM $K^+$ ($E_K$=0 mV) at 0mV: before: 2.9 ± 1.2 nA, after: 5.8 ± 0.9 nA]. Pre-treatment of cells with 5-HT reduced the effects of PKC injections, as did 1-10 nM bath applied concentrations of imipramine, which has been reported to inhibit PKC activation.

Because these effects of PKC are essentially identical to those produced by either associative training or 5-HT application, it is attractive to suppose that 5-HT induced PKC activation is one mechanism by which long-term excitability changes in B cells occur. It is, moreover, a mechanism molecularly distinct from the previously proposed calcium/calmodulin dependent phosphorylation pathway.

1. Alkon et al., Science, 215: 1982. 2. Farley et al., Soc. Neurosci. Abstr., 10: 1984. 3. Farley & Alkon, Soc. Neurosci. Abstr., 9: 1983. 4. Farley, in press: 1985 5. Wu & Farley, Soc. Neurosci. Abstr., 10: 1984. 6. Auerbach et al., this volume. 7. Farley & Auerbach, Biophys. J., 47: 1985. 8. Niedel et al., PNAS, 80: 1983.

Supported by NSF grant BNS-8316707 to Joseph Farley.

227.13 FORSKOLIN-MODULATED MEMBRANE CURRENTS IN APLYSIA TAIL SENSORY NEURONS. D.A. Baxter and J.H. Byrne, Dept. of Physiol. and Cell Biol., Univ. of Texas Med. Sch., Houston, Tx. 77225.

In the sensory neurons that mediate the tail withdrawal reflex serotonin (5-HT) produces a depolarization that is associated with a decrease in their input conductance. Previous studies have shown that 5-HT increases the levels of cAMP in these cells and injection of cAMP or application of the cyclase activator forskolin produces decreases in membrane conductance similar to those produced by 5-HT. To examine further the membrane currents modulated by cAMP we utilized forskolin and voltage-clamp and computer subtraction techniques.

We used surgically isolated sensory cells and conventional two-electrode voltage-clamp techniques and applied 150 ms clamp pulses to various depolarized levels from a holding potential of -70mV (large depolarizations were separated by > 3 min). The procedure was repeated after addition of a saturating concentration of forskolin ($10^{-4}$M) to the bath. Forskolin-modulated currents were obtained by subtracting the responses obtained before and after forskolin addition. At all potentials examined (-40 to +20 mV) forskolin led to the reduction of net outward currents. The kinetics and voltage-dependence of the responses indicated that the forskolin-modulated currents had at least two components. One component, most prominent at low levels of depolarization (-40 to -10mV), was relatively linear with respect to voltage, had an activation time constant (about 7 ms) that was relatively voltage-insensitive, and showed little sign of inactivation during the pulse. The second component was most pronounced at membrane voltages more positive than -10mV. This component exhibited a marked voltage-dependence, had a slower, voltage-dependent activation time constant and displayed inactivation at large depolarizations (> +10 mV). Similar forskolin effects were obtained in ASW with $Co^{++}$ substituted for most of the $Ca^{++}$ (0.1 mM remained). Thus, neither component appears to be due to $Ca^{++}$ influx or nonspecific effects of transmitter release from remaining terminals in the portions of attached neuropil.

The first component has properties consistent with previous descriptions of the S-current (Klein et al, 1982; Siegelbaum et al, 1982) while the second component has not been described previously. We are currently investigating whether the second component is an additional current modulated by forskolin (and presumably cAMP) or a novel component of the S-current itself. These results and others (Boyle et al, 1984; Walsh and Byrne, this volume) indicate that the effects of cAMP on modulating cellular properties of these neurons are rather complex. While complex all the effects may act synergistically to modify synaptic transmission at the sensory-motor neuron synapse. Supported by AFOSR grant 84-0213.

227.14 CYCLIC AMP AND CALCIUM SENSITIVITY OF THE 5-HT RESPONSE IN TAIL SENSORY NEURONS OF APLYSIA. J.H Walsh and J.H. Byrne, Univ. of Texas Med. Sch., Houston, TX. 77225.

Biochemical and voltage clamp studies indicate that 5-HT produces a cAMP-mediated decrease in a resting $K^+$ conductance in sensory neurons involved in the tail withdrawal reflex. The present study was designed to extend the analysis of the cellular and ionic mechanism(s) underlying the 5-HT response of these cells.

To test if each response is produced by a common saturable mechanism, iontophoretic injections of cAMP (0.1 M, 10 ms pulses, 50 Hz for 1 s, 200 nA) were used in conjuction with extracellular pressure microejection of 5-HT (1mM, 0.5 to 1 s pulse) in neurons voltage-clamped at a holding potential of -25 to -37 mV. Injections of cAMP or 5-HT alone produced inward currents associated with decreases in input conductance. Application of 5-HT just prior to a second injection of cAMP resulted in a 75.4 ± 7% (SEM, N = 5) reduction in the amplitude of the cAMP response.

The current elicited by brief microejections of 5-HT onto sensory neurons voltage clamped at a holding potential of -30 to -40 mV appeared to be $Ca^{2+}$-sensitive. The response to 5-HT was attenuated when a variety of $Ca^{2+}$-channel blockers including $Cd^{2+}$ (2 mM), $Co^{2+}$ (30 mM) and $Ni^{2+}$ (15 mM) were added to ASW. In addition, the 5-HT response was also attenuated in ASW solutions where $Ba^{2+}$ was substituted for most of the $Ca^{2+}$ (0.5 mM $Ca^{2+}$ remained). While other interpretations are possible, these results suggest that a component of the 5-HT response may be a modulation of a calcium-activated $K^+$ current ($I_{K,Ca}$). To test this possibility directly $K^+$-currents were elicited by intracellular $Ca^{2+}$ iontophoresis (0.5M $CaCl_2$, 10 ms pulses at 50 Hz for 0.3-2 s, 100 to 1000 nA). The outward currents elicited were 2 to 20 nA in amplitude and persisted for 5 - 25s. Outward currents produced by $Ca^{2+}$ injection were reduced by 31.8 ± 5.6% (SEM, N = 21) when elicited at the peak of the 5-HT response. Upon recovery from the 5-HT response, the outward currents returned to control levels. While control injections of $Ba^{2+}$, $Co^{2+}$, and $Na^+$ (0.5 M) produced no outward current, $Ba^{2+}$ injections (N = 3) produced an inward current associated with a decrease in input conductance, which mimicked both the response to extracellular $Ba^{2+}$ and 5-HT.

These data further support the hypothesis that cAMP acts as a second messenger in the 5-HT response of the tail sensory neurons. In addition, they indicate that 5-HT can modulate $I_{K,Ca}$ as well as the previously described $Ca^{2+}$-insensitive S-current (Siegelbaum et al, 1982).

227.15 CHARYBDOTOXIN SPECIFICALLY BLOCKS CA-ACTIVATED K CONDUCTANCE OF APLYSIA NEURONS. A. Hermann* (Spon: J. Chad). Dept. of Biology, UCLA, Los Angeles, CA 90024.

Charybdotoxin (CTX), a partially purified component of scorpion (Leiurus quinquestriatus) venom, has been recently reported to block potently the large Ca-activated K channels isolated from skeletal muscle and inserted into lipid bilayer (Miller, C. et al., Nature, 313:316, 1985). The present experiments examine the action of CTX on the Ca-activated K current, $I_{K(Ca)}$, of identified neurons in the abdominal ganglion of Aplysia californica. The results show that external CTX blocks the Ca-activated K current, induced by ionophoretic injection of Ca ions into the cells, in a dose dependent manner with a $K_D$ of about 30 nM at a membrane potential of -30 mV. The block is voltage dependent, decreasing with increased positive potential. The time to maximum inhibition is relatively fast, occurring within 1-3 minutes. The action of the venom is reversible after washout, recovery being slower than onset of the block. CTX also suppressed the Ca-dependent hump seen in the N-shaped current-voltage plot of outward current. The venom had little effect on Na or Ca inward currents, the transient K current or the delayed rectifier current. Only high concentrations of the venom (several hundred nM) effect these currents. However, CTX induced an apparent inward shift of steady state current, which was accompanied by a decrease of the membrane resting conductance. The current reversed at about -70 mV, indicating a resting K conductance that is blocked by CTX.

CTX at a concentration of 50-100 nM caused depolarization of spontaneously bursting neurons, and eventually led to repetitive discharge of action potentials. Upon repolarization by current injection, bursting was re-established; however, the number of action potentials was increased and the postburst-hyperpolarization was diminished. These effects were reversible. It is concluded that the bursting discharge is controlled in part by a Ca-activated K conductance.

The results show that CTX is a high-affinity, specific but voltage dependent blocker of the Ca-activated K current in Aplysia neurons.

I am grateful to C. Miller for the gift of CTX. Supported by a Heisenberg grant to A.H., DFG grant SFB 156 and by NSF BNS 83-16417 to R. Eckert.

227.16 CHARYBDOTOXIN (CTX) SELECTIVELY BLOCKS A CALCIUM-MEDIATED POTASSIUM CONDUCTANCE THAT CONTRIBUTES TO THE ACTION POTENTIAL RECORDED OPTICALLY FROM NERVE TERMINALS OF THE FROG NEUROHYPOPHYSIS. A.L. Obaid*, D. Langer* and B.M. Salzberg. Univ. of Pennsylvania, Phila. PA 19104 and Marine Biological Laboratory, Woods Hole, MA 02543.

Voltage-sensitive molecular probes allow one to monitor the action potential in nerve terminals of the frog neurohypophysis by optical means (Salzberg et al., Nature 306:36, 1983). When voltage-sensitive Na and K channels are blocked with Tetrodotoxin (TTX) and Tetraethylammonium (TEA), direct field stimulation of the nerve terminals evokes regenerative responses (Obaid et al.. J. Gen. Physiol. 85:481, 1985) that result entirely from a voltage-sensitive calcium influx. Even in the absence of TTX and TEA, this calcium component of the normal action potential is probably sufficient to mediate hormone release. Both the normal action potential and the calcium response exhibit prominent afterhyperpolarizations that result from a calcium-mediated increase in potassium conductance.

Charybdotoxin (CTX), a protein toxin derived from scorpion venom, blocks calcium-sensitive potassium channels from rat skeletal muscle in planar lipid bilayers (Miller et al., Nature 313:316, 1985). We report here that CTX (50 nM) specifically blocks the component of the optical signal that reflects the calcium-mediated potassium conductance in the nerve terminals of the frog neurohypophysis. This block is apparent in the normal action potential and in the calcium response. In both instances, the undershoot is eliminated and the width and height of the action potential are increased.

Apamin, a low molecular weight fraction from bee venom, is reported to block calcium-mediated potassium conductances in several preparations. As supplied by Sigma (A 5775; lot 104F0166), it yields two peaks by HPLC and has a purity of approx. 50%. This Apamin also blocks the calcium-mediated potassium conductance in the nerve terminals of the frog, but at concentrations ten times higher than CTX, with longer incubation, and without the specificity of CTX. For example we find a reduction in the height of the action potential and in its maximum rate of rise, as though voltage dependent Na channels are also blocked by this material.

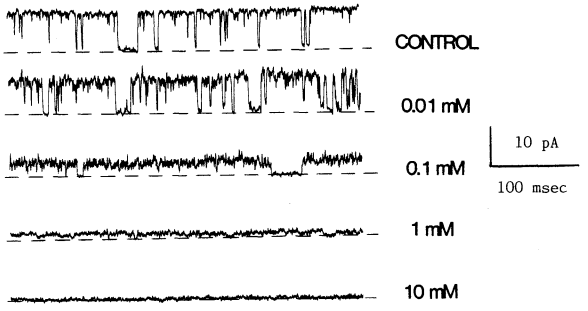
We are grateful to Chris Miller for his generous gift of CTX. Supported by USPHS grant NS 16824.

227.17 ASYMMETRICAL SENSITIVITY OF TETRAETHYLAMMONIUM BLOCKADE OF CALCIUM-ACTIVATED POTASSIUM CHANNELS IN CLONAL ANTERIOR PITUITARY CELLS. B. S. Wong[1] and M. Adler[2]. [1]Dept. of Physiology, Baylor College of Dentistry, Dallas, TX 75246, [2]Neurotoxicology Branch, USAMRICD, APG, MD 21010.

The effects of tetraethylammonium (TEA) ions on blocking single calcium-activated potassium channels were studied in excised outside-out and inside-out membrane patches from the mouse anterior pituitary cell line AtT-20/D16-16 with the patch-clamp technique. Both externally and internally applied TEA resulted in a decrease in the single-channel current. The dissociation constant at zero voltage for the TEA-receptor complex was found to be 52.2 mM and 0.08 mM for external and internal TEA, respectively This is in contrast to single-channel studies in other cell types which demonstrated external TEA as being more effective than internal TEA in blocking ion flows through these channels. The Hill plot analysis of the dose response data yielded a slope of 0.92, indicating a one-to-one stoichiometry for TEA-receptor binding. The blockade by TEA showed little voltage sensitivity over the membrane potential range studied, was independent of the direction of current flow, and could be fully reversed by wash-out in drug-free solution. The results suggest the presence of TEA receptors on both the external and internal membrane surfaces but with different binding affinities. Occupancy of either site by TEA leads to a decrease in the single-channel conductance of calcium-activated potassium channels. The asymmetrical sensitivity to TEA, however, suggests that the end regions of these channels may differ in different cell types.

(Supported in part by BCD Research Fund and the American Heart Association, Texas Affiliate.)

EFFECTS OF INTERNAL TEA




227.18 THE CALCIUM- AND VOLTAGE-DEPENDENT $K^+$ CHANNEL OF CLONAL ANTERIOR PITUITARY CELLS: PHARMACOLOGICAL MANIPULATION AND ROLE IN ACTION POTENTIAL REPOLARIZATION. M.A. Rogawski, B. Dufy and J.L. Barker. Laboratory of Neurophysiology, NINCDS, NIH, Bethesda, MD 20205.

Under voltage clamp, a $Ca^{2+}$-dependent $K^+$ conductance that activates slowly following depolarization and maintains a plateau with persistent depolarization is recorded in $GH_3$ cells (a rat clonal pituitary cell line). To investigate the functional role of this conductance in the firing behavior of these cells, we characterized the properties and pharmacological sensitivity of the underlying single $K^+$ channels in intact and excised outside-out membrane patches. In the cell attached configuration, patch clamp recordings (5.5 mM external $K^+$) revealed large conductance, non-inactivating outward single channel currents whose amplitude varied linearly with membrane potential in the range 0-70 mV depolarized from rest. Often, channel openings occurred simultaneously with action potentials recorded by the patch electrode. The single channel conductance was 55-75 pS and the extrapolated reversal potential was 16 mV hyperpolarized to rest. Burst openings were infrequent and brief at resting potential, but the number of openings increased markedly as the patch was depolarized. The mean burst lifetime also increased with depolarization (in a sigmoidal fashion from 2-11 msec). When the concentration of $K^+$ in the patch pipette was raised, the reversal potential shifted in the depolarized direction as predicted by the Nernst equation for a $K^+$ selective membrane pore. With symmetrical $K^+$ across the membrane, the single channel conductance was approximately double that observed with the normal $K^+$ gradient. External application of $Ba^{2+}$ (10 mM) to the whole cell (but not to the patch) caused a failure of channel opening. In contrast, quinine (5 mM) caused high frequency flickering of the channel. These channel blockers seem to enter the cell and interfere with channel activity from the inside. Tetraethylammonium (TEA; 20 mM) and apamin (5 μM) applied in this manner were without effect. A23187 (10 μM; a $Ca^{2+}$ ionophore) and 4-aminopyridine (10 mM) caused a stimulation of channel activity. The $Ca^{2+}$ sensitivity of the channel was studied in excised outside-out membrane patches. Channel opening was infrequent with 1 μM free internal $Ca^{2+}$ but increased markedly as the concentration was raised 50-fold. TEA (but not apamin) applied to the external membrane face in the outside-out configuration caused a powerful blockade of channel activity. In current clamp recordings, only the specific blockers of the channel ($Ba^{2+}$, TEA, quinine) caused a major broadening of action potentials; other $K^+$ channel blockers that did not interfere with these channels (4-AP, apamin) had little effect on spikes. We conclude that the $Ca^{2+}$- and voltage-dependent $K^+$ channel in pituitary cells plays an important role in the repolarization of the $Ca^{2+}$-dependent action potentials of these cells.

## SPECIAL LECTURE

228 SPECIAL LECTURE. GENE EXPRESSION IN THE MAMMALIAN BRAIN. J.G. Sutcliffe, Research Institute of Scripps Clinic.

About 30,000 polyA$^+$ mRNAs are expressed in the adult rat brain, and more than 50% of these by mass are specific to the brain. Hence, cDNA cloning can easily provide copies of large numbers of brain specific mRNAs for analysis. By DNA sequence analysis of full-length copies of brain specific mRNAs, we generate the amino acid sequences of putative brain specific proteins. Computer comparisons with known protein sequences provides some information about possible function. We synthesize a series of non-overlapping peptides corresponding to short regions within the putative sequence and use antisera to the synthetic peptides to identify the product of the brain specific mRNA. The antisera reveal anatomical and subcellular distributions of each protein, and this information combined with the primary sequence can suggest possible biochemical functions. Thus far these studies have led to the description of a precursor of a family of probable neuropeptides found predominantly in olfactory somatosensory and limbic systems that is the product of a gene which is very closely linked (and may be) the quiverer locus; the two major mRNAs for brain myelin proteolipid protein transcribed from a single gene on the X chromosome; and a large polymorphic family of mRNAs, many of which are specific to the nervous system.

The 5-10,000 genes expressed exclusively in adult rat neurons as polyA$^+$ messenger RNA (mRNA) contain a common element called an ID sequence within their noncoding introns. This element is transcribed specifically in neurons by RNA polymerase III, the enzyme which generates tRNAs and other small RNAs. This transcription begins around the time of birth, and its tissue specificity results from a transcriptional protein exclusive to brain. Subsequently the adult brain specific genes which contain the ID element within their introns are transcribed by RNA polymerase II, the enzyme which generates mRNAs. We believe the Pol III transcription events are equivalent to the process of determination in which the genes which can be expressed in the adult nervous system are selected. Further events during development and experience are necessary for the expression of each particular gene in particular sets of neurons. The ID elements act as enhancers (positive activators) of gene expression when fused to genes whose products can be assayed in transfection experiments. Thus they appear to be neuronal specific regulators of gene expression.

229 VISUAL MOTION PROCESSING IN CEREBRAL CORTEX. R. H. Wurtz, National Eye Institute (Chairperson); S. Ullman, Massachusetts Institute of Technology; J. A. Movshon, New York Univ.; W. T. Newsome, State Univ. of New York at Stony Brook; V. B. Mountcastle, Johns Hopkins Univ. School of Medicine.

This symposium summarizes recent advances made in the analysis of visual motion processing in the neocortex of primates. Emphasis is on the functional contribution of cortical areas rather than their anatomical or physiological organization. The introduction by R. H. Wurtz will outline the systems within the primate brain related to motion processing and emphasize the importance of cerebral cortical processing in primates in comparison to other mammals. S. Ullman will review the main problems involved in the processing of visual motion. The problem of measuring visual motion will serve to illustrate recent fruitful collaboration between physiological and computational research. The subsequent presentations will describe investigations of neural activity at three levels of neocortex: the striate cortex, where motion processing is first observed in the primate neocortex, the middle temporal area (MT) in the prestriate cortex, which is probably devoted largely to motion processing, and the parietal cortex, which probably represents one of the highest levels of processing within the cerebral cortex. J. A. Movshon will compare the type of physiological processing occurring in the striate cortex, with that in area MT. Neurons in the striate cortex respond only to the motion of the oriented elements of a complex two-dimensional pattern while cells in area MT integrate signals concerning the movement of the pattern as a whole. Support for the idea that MT is specialized for the analysis of motion will be provided by W. T. Newsome. Chemical lesions of MT reduce a monkey's ability to make pursuit and saccadic eye movements to moving visual targets but not to stationary visual targets. V. B. Mountcastle will describe more complex visual motion processing and the organization of motion directionalities in the parietal cortex.

230 SYMPOSIUM. BIOLOGY OF ISOLATED BRAIN CAPILLARY ENDOTHELIA: AN EMERGING FIELD IN NEUROSCIENCE. W.M. Pardridge, UCLA School of Medicine (Chairman); G.W. Goldstein, University of Michigan Medical Center; P.A. Cancilla*, UCLA School of Medicine; M.A. Moskowitz, Harvard Medical School; F.P. White and G.R. Dutton, Washington State University College of Veterinary Medicine and University of Iowa School of Medicine.

Brain capillary endothelia in virtually all vertebrates make up the blood-brain barrier (BBB) in vivo. Within the context of a barrier paradigm, the brain capillary endothelial cell has generally been viewed as serving a passive and protective role in brain metabolism and function. However, the techniques of cell biology have generated a new paradigm of brain capillary endothelial function, which visualizes dynamic interactions between capillary endothelia and neurons and glia. For example, glial cells are now believed to release as yet undefined trophic factors that are sequestered by capillary endothelia to initiate the genetic expression of unique biochemical properties in the brain capillary endothelial cell. The trophic factors released by brain and the gene products of the endothelial cell are now amenable to investigation using primary culture model systems of brain capillary endothelia. The development of monoclonal antibodies that are specific for surface antigens present in brain microvascular elements may also be employed to investigate the expression in brain capillaries of proteins unique to that cell. In addition to the long-term regulation by brain of gene expression in brain capillary endothelia, model systems now available also make accessible the study of acute signal transduction phenomena in brain capillary endothelia that may be related to the regulation of cerebral blood flow and metabolism on a minute-to-minute basis. Phospholipid-mediated signal transduction pathways have been identified in brain endothelia and the activity of protein phosphorylation pathways in isolated brain capillaries have been found to be nearly as active as that observed in synaptosomal membranes. Finally, peptide receptor transport systems have been identified using isolated brain capillaries and an understanding of the receptor-mediated transport of circulating peptides through the brain capillary endothelia may lead to new strategies for peptide delivery to brain.

## ACTION POTENTIALS AND ION CHANNELS VI

231.1 A NEW METHOD FOR CHANGING INTRACELLULAR CALCIUM CONCENTRATION. R. S. Zucker and R. Y. Tsien*. Dept. of Physiology-Anatomy, Univ. of Calif., Berkeley, CA 94720.

A photolabile calcium chelator has been developed as a tool for rapidly increasing intracellular calcium concentration in single cells. A new class of substance has been synthesized, comprised of 2-nitrobenzhydrol derivatives of BAPTA (1,2-bis(2-aminophenoxy)ethane N,N,N',N'-tetraacetic acid) chelator. One member of this class, designated nitr-2, has been characterized chemically and used physiologically to control intracellular calcium in single neurons. Nitr-2 chelates a single calcium ion with a dissociation constant of 160 nM, measured at an ionic strength of 0.1 M. Ultraviolet light photoisomerizes the 2-nitrobenzhydrol form to a 2-nitrosobenzophenone, with a quantum efficiency of 0.03 for a wavelength of 365 nm, which is the absorbance peak of the difference spectrum for the two forms. Ultraviolet light also photoisomerizes the $Ca^{2+}$-complex of nitr-2 to a $Ca^{2+}$-nitrosobenzophenone with a quantum efficiency of 0.1-0.2. The nitrosobenzophenone has a markedly reduced affinity for calcium ($K_D$=7 μM). Thus exposure of $Ca^{2+}$-nitr-2 to a flash of ultraviolet light causes the release of "caged calcium" from the chelator, with a reaction time constant of about 250 msec. The amount of calcium released depends in a linear manner on the light intensity and duration, for flash energies that isomerize a small fraction of the nitr-2.

Single neurons, about 250 μm in diameter, from the abdominal ganglion of Aplysia californica, were microinjected by pressure with about 10 mM nitr-2, 75% in the calcium form. Since the $K_D$ of nitr-2 for calcium is similar to the resting free calcium level in cytoplasm, the latter is little affected by the injected buffer. Membrane currents were measured under voltage clamp. Calcium-activated potassium current and calcium-activated nonspecific cation current were isolated pharmacologically (using 50 mM tetraethylammonium to block $I_{K(Ca)}$). Both currents could be elicited by focusing light flashes from a xenon arc lamp (about 225 J in 2 ms) onto the filled cell. Both currents reversed at the potentials expected from their ionic selectivities, and reached a maximum at about 25-40 ms. Repeated flashes elicited identical responses. Response amplitude was graded with light intensity.

In summary, photo-isomerization of nitrobenzyl BAPTA derivatives provides a new way to release controlled amounts of calcium relatively uniformly throughout the cell's interior. This should be a valuable tool in the study of calcium-regulated processes. Supported by NIH Grants NS 15114 to R.S.Z., and GM 31004, EY 04372, and a Searle Scholars Award to R.Y.T.

231.2 SINGLE CALCIUM-ACTIVATED CHANNELS WITH HIGH CA-SENSITIVITY AND SMALL CONDUCTANCE IN CULTURED RAT SKELETAL MUSCLE. A. L. Blatz* and K. L. Magleby. Dept. of Physiology and Biophysics, University of Miami, Miami, FL 33101.

Ca-activated K currents occur in most muscle and nerve cells. The channels underlying these currents often give rise to slow hyperpolarizations following action potentials and can serve to regulate low frequency repetitive firing in neurons and cultured skeletal muscle. Romey & Lazdunski (BBRC 118, 669-674, 1984) have shown that the long-lasting afterhyperpolarization in cultured skeletal muscle is not caused by the large conductance (250-300 pS) Ca-activated K channel (BK channel) but is due to another Ca-activated conductance that can be blocked by micromolar amounts of the bee venom apamin. While searching for the apamin blockable Ca-activated channel in cultured skeletal muscle using the giga-seal patch clamp technique we found three additional Ca-activated channels with conductances of about 4, 12 and 32 pS, all considerably less than the BK channel. All of these channels had a greater Ca sensitivity at negative membrane potentials than the BK channel. In most of the membrane patches examined the small conductance Ca-activated channels were present in such high density that the major effect of increasing internal Ca from 1 nM to 100 nM was an increaed inward steady-state current and a dramatic increase in the current fluctuations, with no clear single channel open shut transitions apparent (-40 mV). In some patches, however, the small conductance Ca-activated channels were present at a low enough density for individual single channel currents to be observed. The channels with 12 pS conductance in symmetric 140 $KCH_3SO_4$ were about ten times more sensitive to internal Ca than the BK channels in the same patch. The 12 pS channels were not observed in four patches with 0.75 uM apamin in the external solution (10 uM $Ca_i$). At a holding potential of -40 mV the 12 pS channels were open 6% of the time at 0.1 uM $Ca_i$ and 60% of the time at 10 uM $Ca_i$. The 32 pS channels were active even when apamin was added to the external solution. The small 4 pS channels were apparently the most Ca sensitive, as they would become active before the others when $[Ca]_i$ was increased during a solution exchange. In these experiments K was the major charge carrier through these channels. (The internal solutions contained (mM): 140 KCl or $KCH_3SO_4$, 5 TES buffer (pH 7.2), 0.5 EGTA and sufficient $CaCl_2$ for the specified free $[Ca^{2+}]_i$. External solutions were identical except that $[Ca^{2+}]_i$ was usually 10 nM). We have not yet determined the selectivity of these channels to other cations such as Na. Supported by grant AM 32805 from the NIH and a grant from the Muscular Dystrophy Association.

231.3 CALCIUM CURRENTS IN DEVELOPING MAMMALIAN SKELETAL MUSCLE CELLS. K.G. Beam* and C.M. Knudson* (SPON: L. Marshall) Dept. of Physiol. Biophys., Univ. of Iowa, Iowa City, IA 52242.

We have been using the whole-cell variant of the patch clamp technique to examine inward ionic currents in developing skeletal muscle. Outward currents were blocked with $Cs^+$ in the patch electrode and tetraethylammonium ion in the bath. Two preparations were used: rat and mouse skeletal myotubes grown in primary tissue culture and enzymatically dissociated fibers of flexor digitorum brevis (FDB) muscles acutely removed from 0-25 day old rats.

Three distinct inward currents are present in skeletal myotubes. The largest and most rapidly activated of these is a sodium current. To study calcium currents in isolation, $I_{Na}$ was eliminated by adding TTX (10 uM) and removing extracellular sodium. Elevated extracellular Ca (50 mM) was used in most experiments, both to accentuate the calcium currents and to improve electrode sealing. Under these conditions, depolarizing from the holding potential (-90 mV) to potentials in the range -30 to 0 mV activated a transient inward current ($I_{fast}$) that peaked in 15-30 ms and completely inactivated within about 100 ms. Stronger depolarizations (+10 mV and above) activated a second, slower inward current ($I_{slow}$) that peaked in about 100 ms and inactivated little during a 200 ms test pulse. $Cd^{+2}$ (0.1-0.2 mM) blocked both currents, with the slow current being more sensitive. Holding at a potential of -30 mV completely inactivated $I_{fast}$ with little effect on $I_{slow}$.

A slow calcium current resembling $I_{slow}$ in myotubes has been described for muscle fibers of adult frogs and rats. Further, we observed that such a current is present in FDB fibers of both 0-1 day and 25 day rats. Thus, a current like $I_{slow}$ appears to be present in muscle fibers throughout much of their development.

A current with kinetics resembling $I_{fast}$ of myotubes has not been described for adult rat or frog. In FDB fibers of 0-1 day rats a current like $I_{fast}$ of myotubes is present for the same range of test potentials. In 25 day FDB fibers, such potentials cause a rapidly activating current that shows little inactivation over 200 ms. A current with properties like those of $I_{fast}$ in 25 day FDB fibers has recently been described for muscle fibers of adult frogs (Cota, G.D. & Stefani, E., Biophys J., 47:65a, 1985).

Supported by MDA, and by NIH grants NS-14901 and RCDA NS-00840 to KGB.

231.4

**WITHDRAWN**

231.5 MACROSCOPIC CALCIUM CURRENTS IN ACUTELY-EXPOSED GRANULE CELLS FROM ADULT HIPPOCAMPUS. R. Gray and D. Johnston, Prog. in Neuroscience and Dept. of Neurology, Baylor Coll. of Med., Houston, Tx. 77030.

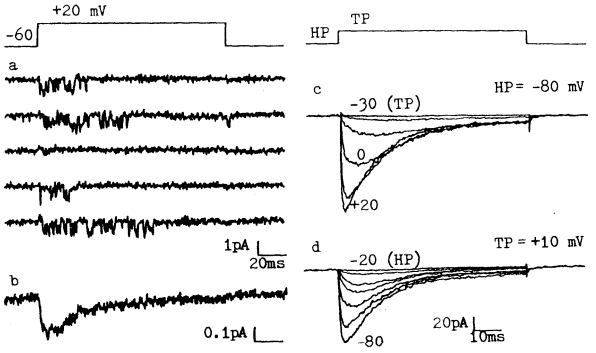
It has been shown recently that β-adrenergic agonists are able to modulate long-term potentiation (LTP) of synaptic transmission at the granule cell mossy fiber to CA3 synapse in the hippocampus (Hopkins & Johnston, Science 226:350). We were interested in the possibility that β-agonists may have direct effects on ionic currents that might underly this modulation of LTP. β-adrenergic agonists have been reported to affect both Ca and K currents in a variety of cell types. We have begun to investigate the properties of isolated Ca currents in acutely-exposed granule cells from the adult guinea pig hippocampal formation.

Hippocampi were dissected from adult guinea pigs and 300 μm transverse slices were made. The slices were then treated with proteolytic enzymes and gently agitated to expose the neural somata (Gray & Johnston, J. Neurophysiol., in press). Granule cells maintained at 15-23°C were voltage-clamped using the giga-seal whole-cell clamp technique. Intracellular solutions included tetraethylammonium (TEA), 3,4-diaminopyridine (3,4-DAP), and Cs to block K currents. The bath solution also included TEA and 3,4-DAP in addition to tetrodotoxin and 2 mM Ca. Using trains of brief voltage steps superimposed on longer depolarizing step commands, we were unable to detect any activation of outward currents in the presence of these K channel blockers.

Isolated Ca currents observed under these conditions showed voltage-dependent activation consistent with $m^2$ kinetics and slow inactivation (accompanied by a conductance decrease) during step voltage commands. At 23°C the time constant for activation of the Ca current varied from 4.5 ms with commands to -20 mV to 1.0 ms with commands to +20 mV. The Ca tail currents could be fit by two exponentials with mean values of 200 μsec and 2 msec. Washout of the Ca current usually occured within 30 min of recording, but could be slowed by cooling to temperatures below 20°C. Application of norepinephrine (1-5 μM) or isoproterenol (0.5-2 μM) from a puffer pipette placed near the clamped cell body was found to cause an increase in the peak amplitude of the Ca current without changes in activation or tail current kinetics. Preliminary computer simulations using compartmental modeling methods (Johnston & Brown, J. Neurophysiol. 50:464; Carnevale et al., Biophys. J. 47:66a) suggest adequate space clamp conditions in the cells chosen for analysis. The possibility that the observed enhancement of a Ca current by β-adrenergic agonists plays a role in synaptic plasticity is currently under investigation. (Supported by NIH NS15772, NS11535, a McKnight Neurosci. Devel. Award, USAMRDC DAMD17-82-C-2254, and AFOSR-85-0178.)

231.6 KINETIC AND PHARMACOLOGICAL PROPERTIES DISTINGUISH N-TYPE CALCIUM CHANNELS FROM OTHER TYPES OF CALCIUM CHANNEL IN CHICK SENSORY NEURONS. A.P. Fox, M.C. Nowycky+ and R.W. Tsien, Dept. of Physiology, Yale Univ. Sch. Med., New Haven CT 06510 and +Dept. of Anatomy, Medical College of Penn., Philadelphia PA 19219.

We find three types of calcium channel on cell bodies of chick dorsal root ganglion cells with quite different conductances with 110 mM Ba in the patch pipette. A ~25 pS channel (L) accounts for long-lasting currents activated at strong depolarizations; an ~8 pS channel (T) underlies transient currents activated at weak depolarizations; a ~13 pS channel (N) with distinctive properties is described here. N-type channel activity (left column) activates with strong test pulses, unlike T-type channels. Unlike L-type channels, it also requires negative holding potentials (HP) for repriming, and shows a fairly rapid time-dependent inactivation, seen in individual sweeps (a) or an averaged current record (b). We often find patches containing >100 N-type Ca channels and few if any T- or L- type channels. These are useful for studying activation or inactivation kinetics (c,d). With test pulses from HP=-80 mV (c), N-type channel activation becomes clear at -20 mV (T-type activity appears at -50 mV and L-type activity appears at 0 mV). When channel availability is tested with pulses to +10 mV (d), inactivation is removed between HP=-20 and -80 mV; in contrast, T-type channels are totally inactivated at HP=-40 while L-type channels are partly available even at HP=0 mV. Unlike L-, N- and T-type channels fail to respond to 5 uM Bay K 8644. Conversely, L- and N-type channels are almost fully blocked by 50 uM Cd, while T-type channels are not. All of these results support the idea of three distinct channel types.



231.7 $^{3}$H-NITRENDIPINE BINDING SITES CORRELATE WITH DIHYDROPYRIDINE-SENSITIVE CALCIUM UPTAKE IN CULTURES OF CEREBELLAR GRANULE CELLS AND NG108-15 HYBRID CELLS. E. Carboni* and W.J. Wojcik (SPON: R. Levy). Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hosp., Washington, D.C. 20032.

Calcium influx through voltage dependent calcium channels is important in coupling excitation and contraction in cardiac and smooth muscle and in releasing neurotransmitters. In the last few years, the voltage sensitive calcium channel antagonists have been tested in the above systems. Even though they have recognized effects in cardiac and smooth muscle preparations, some controversy exists about their action in neuronal systems. In brain membrane preparation, $^{3}$H-Nitrendipine binding has been well characterized, demonstrating the presence of high affinity sites. Despite the presence of these high affinity binding sites, it has been difficult to show any antagonism of calcium uptake into neuronal tissue by dihydropyridines as nitrendipine or stimulation of uptake by the agonist BAY K 8644.

We have investigated the voltage sensitive calcium channels by measuring $^{45}Ca^{++}$ uptake and $^{3}$H-nitrendipine binding in primary cultures of cerebellar granule cells and in neuroblastoma-glioma NG 108-15 cells. In membranes prepared from 8 day old cultures of granule cells, we found a high affinity $^{3}$H-nitrendipine binding site ($K_D$= 0,71 nM, Bmax= 73.3 fmol/mg prot.), which was displaced by other dihydropyridines with $IC_{50}$'s in the nanomolar range. In those studies involving $^{45}Ca^{++}$ uptake in the intact cells, nitrendipine (100 nM) was able to inhibit the 60 mM $K^+$ induced $^{45}Ca^{++}$ uptake by 60%. This depolarizing concentration of $K^+$ stimulated $^{45}Ca^{++}$ uptake by three times. BAY K 8644 (100 nM) stimulated calcium uptake by five fold in the presence of 60 mM $K^+$ and nitrendipine (100 nM) was able to counteract this stimulation by 75%. BAY K 8644 did not affect calcium uptake with physiological concentrations of $K^+$. Veratridine (50 uM), was able to increase calcium uptake at 5 mM $K^+$ by 2.5 times and BAY K 8644 (100 nM) further increased calcium uptake by 4.2 times. Nitrendipine (100 nM) was able to antagonize the effects of veratridine and veratridine plus BAY K 8644.

In NG108-15 cells, 60 mM $K^+$ stimulated $^{45}Ca^{++}$ uptake by 1.8 times while BAY K 8644 (100 nM) increased uptake by 2.5 times. In this preparation, nitrendipine (100 nM) completely antagonized the calcium uptake induced by 60 mM $K^+$ and by 75% in presence of 60 mM $K^+$ plus BAY K 8644. $^{3}$H-Nitrendipine binding in membrane preparation of these cells gave a $K_D$ = 1.1 nM and a Bmax = 5.76 fmol/mg prot.

In conclusion our data, support the presence of functional 1,4-dihidropyridine sites associated with calcium channels in primary cultures of cerebellar granule cells and in neuronally derived neuroblastoma-glioma hybrid cells.

231.8 VOLTAGE-DEPENDENT CALCIUM CHANNELS IN XENOPUS OOCYTES INJECTED WITH EXOGENOUS mRNA. N. Dascal*, T. P. Snutch*, H. Lubbert*, N. R. Davidson* and H. A. Lester. Divisions of Biology and Chemistry, Caltech, Pasadena CA 91125.

As reported earlier by other groups (e.g., Gundersen et al., Nature, 308:421; Houamed et al., Nature, 310:318), the injection of mRNA from brain resulted in the appearance of ion channels activated by agonist (GABA, kainate, serotonin) and voltage (Na, K, Cl) in oocyte's membrane. 2-3 days after injection of brain, muscle and heart RNA, an additional depolarization-activated current was resolved with 40 mM external Ba, 0 external Cl, and intracellular TEA injections. This current was blocked by 0.1 mM Cd or 2mM Co but not by TTX. The peak amplitude was as high as 150 nA and correlated well with that of the Ca-activated Cl current observed in the same cells in normal physiological solution. We conclude that the Ba current flows through voltage-dependent calcium channels (VDC's).

The Ba currents from heart RNA peak at +10 mV and show little decline during a 1-s depolarization; brain and muscle RNA induces Ba currents with a peak at 0 mV and half-decay times of about 70-80 and 100 msec, respectively. Ba currents in heart- and brain mRNA-injected oocytes were substantially enhanced and prolonged by exposure to the adenylate cyclase activator, forskolin. Similar enhancement was observed for several minutes after direct intracellular pressure injection of cyclic AMP; this effect was repeated several times in a given oocyte.

By comparing results with total, poly(A)$^-$ and poly(A)$^+$ RNAs, we conclude that the calcium channels are synthesized from poly(A)$^+$ mRNA. RNA was injected from rats of various ages; the synthesis of heart VDC is maximal between 6 and 8 days postnatal. Supported by GM-29836, GM-10991, AHA, Bantrell Foundation, Deutsche Forschungsemeinschaft, and Fulbright Award.

231.9 CAN VOLTAGE-SENSITIVE CALCIUM CHANNELS OF THE RAT CHROMAFFIN CELLS BE OPENED AT THE RESTING MEMBRANE POTENTIAL? T.D. Wakade*, R.K. Malhotra*, T.R. Sharma* and Arun R. Wakade.(SPON: R. Margolis). Department of Pharmacology, State University of New York, Downstate Medical Center, Brooklyn, NY 11203.

A number of procedures and agents have been known to induce secretion of catecholamines (CA) from the adrenal medullary cells. The increase in intracellular Ca resulting from opening of potential-sensitive or receptor-linked Ca channels is primarily responsible for triggering exocytotic secretion of CA. We describe here an entirely new approach to evoke enormous secretion of CA in a Ca-dependent manner. The method offers a new tool to study the behavior of potential-sensitive Ca channels of the chromaffin cells and their role in the secretion of CA.

The experiments were carried out on the isolated perfused adrenal gland of the rat (Wakade, J. Physiol. 313: 463, 1981) and the findings were as follows: Perfusion of the adrenal gland with hypertonic solution (HS; 275 mM NaCl-Krebs solution) caused an increase (68 vs. 180 pg/mg) in the uptake of $^{3}$H-tetraphenylphosphonium (TPP), a lipophilic marker used to detect changes in the membrane potential of chromaffin cells. (The uptake of TPP has been shown to be inversely related to the membrane potential.) Reperfusion with normal solution (NS) reduced the uptake of TPP to the control level (180 vs. 80 pg/mg). Although during prolonged perfusion with HS there was no change in the rate of secretion of CA, it increased from about 1 ng to 400 ng/min after switchover from HS to NS, and the secretory response persisted up to 40 min at the level of 80 ng/min. The net uptake of $Ca^{45}$ in the adrenal medulla was markedly enhanced (6-fold) when the perfusate was changed from HS to NS. The increase in the net uptake of $Ca^{45}$ was seen up to 120 min after the changeover of solutions. The increase in the uptake of $Ca^{45}$ was always associated with an increase in the secretion of CA. The secretion evoked by the switchover of HS to NS was reduced in the presence of Ca channel-blockers and by Ca deprivation. If the adrenal gland was perfused with HS made by adding 156 mM choline chloride, arginine hydrochloride, or 312 mM sucrose to Krebs solution, essentially the same phenomenon of massive secretion of CA was observed after switchover to NS. Chronic splanchnectomy, antagonists of nicotine and muscarine receptors or tetrodotoxin did not affect the secretion of CA evoked by switchover from HS to NS. It is concluded that the threshold value for opening of Ca channels of rat chromaffin cells is not fixed at a critical membrane potential level and probably drifts in relation to the resting membrane potential. Furthermore, a relative change in membrane potential rather than the absolute change is sufficient to opening of these Ca channels.

(Supported by NIH Grants Nos. HL18601, HL22170, and NSF Grant No. BNS 8409685.)

231.10 REGULATION OF NOREPINEPHRINE RELEASE FROM PC12 PHEOCHROMOCYTOMA CELLS BY CALCIUM CHANNEL MODULATORS. S. Kongsamut*, R.M. Nonacs* and R.J. Miller*. Dept. of Pharmacological and Physiological Sciences, University of Chicago, Chicago, IL 60637.

Evoked neurotransmitter release from neurones has usually been found to be insensitive to dihydropyridines (DHP) and other calcium channel blocking drugs. PC12 pheochromocytoma cells can be grown to resemble endocrine cells or neurones depending on whether nerve growth factor (NGF) is added. We therefore investigated the pharmacology of transmitter release from several clones of PC12 cells (PC12T, S or P) grown with or without NGF (50ng/ml).

NGF untreated PC12T cells responded to increasing depolarization with increasing $[K^+]_o$ by releasing preloaded [$^{3}$H]-norepinephrine (NE). Half maximal release of NE occurred at 40mM $[K^+]_o$. The evoked release of NE could be blocked by divalent cations and organic calcium channel blockers. Nitrendipine and BAY K8644 blocked and enhanced depolarization induced release respectively in a dose dependent fashion. In addition, the (+) and (-) isomers of the novel DHP 202-791 enhanced and inhibited [$^{3}$H]-NE release respectively. NGF treatment of PC12T cells resulted in the appearance of short stubby neurites. However, evoked $^{3}$H-NE release from such cells remained sensitive to DHP's. When grown without NGF, [$^{3}$H]-NE release from PC12P cells was also sensitive to DHP's. NGF treatment of PC12P cells resulted in much more extensive morphological differentiation and the appearance of long cell processes. However, evoked [$^{3}$H]-NE release remained DHP sensitive. NGF treatment of PC12S cells resulted in little morphological differentiation and no neurite formation. Either with or without NGF release was DHP sensitive. Interestingly, on long term culture the DHP sensitivity of PC12S cells declined so that eventually evoked release of [$^{3}$H]-NE became completely insensitive to DHP's. This was true for both NGF treated and untreated cells. Treatment of PC12T and S cells with NGF resulted in the appearance of high affinity binding sites for [$^{3}$H]-DAla$^2$,DLeu$^5$-enkephalin [DADLE]. In the case of PC12T cells, binding increased over a two week treatment period from nearly undetectable levels to 20 fmol/mg protein. The $K_D$ was 1.6nM. We are currently examining the opiate sensitivity of transmitter release from these cells.

Treatment of PC12T cells with agents that increased [cAMP] such as forskolin or adenosine augmented evoked [$^{3}$H]-NE release. The extra release produced by these agents was also sensitive to DHP's.

We conclude, (1) that in PC12 cells at any rate the DHP sensitivity of transmitter release does not necessarily correlate with neuronal growth mode and (2) that cAMP enhances [$^{3}$H]-NE release from PC12 cells utilizing a DHP sensitive pathway. We hypothesize that the DHP sensitive calcium channel in neurones may be regulated by cAMP as in the heart and that this may be a mechanism for the modulation of transmitter release from neurones possessing such channels.

231.11 LEPTINOTARSIN-d OPENS RAT BRAIN CALCIUM CHANNELS. M.L. Koenig, J. Connor, T. Hsaio*, and W.O. McClure. Section of Neurobiology, USC, Los Angeles, CA 90089-0371; Dept. Mol. Biophysics, AT&T Bell Laboratories, 600 Mountain Ave., Murray Hill, NJ 07974; Dept. Biol., Utah State Univ., Logan, UT 84322.

Leptinotarsin-d (LPT), a 45,000 dalton protein purified from hemolymph of the Colorado potato beetle, has been shown to effect neurotransmitter release from rat brain synaptosomes by opening presynaptic calcium channels. We have found that LPT has no effect on the calcium channels of Paramecium caudatum, squid optic lobes, cultured PC12 cells, or identified neurons in several gastropod molluscs. The toxin is a potent activator of mammalian calcium channels however (Kd for Ca-dependent release = 3.8 nM), and seems to act with maximal effect on channels derived from the mammalian central nervous system (CNS).

Since a number of calcium channel blockers bind with high affinity to rat brain membranes, presumably to calcium channels, we have investigated the possibility that LPT-stimulated release might be inhibited by preincubation of the synaptosomes with these antagonists. At concentrations of 0.1 to 100 uM, neither nitrendipine nor nifedipine can block LPT-stimulated release of acetylcholine (ACh) from rat brain synaptosomes. Concentrations of verapamil equal to or greater than 10 uM only marginally inhibit (10-15%) the release of ACh in the same system.

We have also studied the effect of LPT on calcium flux across the membranes of CNS neurons by using the calcium indicator dye FURA-2 and digital imaging techniques. Addition of 5 nM LPT to a heterogeneous culture of rat diencephalon cells results in a dramatic increase in the intracellular calcium concentration of some but not all of the cells. The fact that not all cells are affected provides further evidence that LPT is neither permeabilizing cell membranes nor altering the normal calcium exchange mechanisms. Influx of calcium into the affected cells becomes maximal after 8 minutes and cannot be eliminated by extensive washing. If the same cells are washed with a saline solution containing 10 uM nifedipine, the intracellular calcium concentration is rapidly restored to its normal low resting level (<0.1 uM). Thus nifedipine, if added subsequent to LPT exposure, is able to reverse the effect of LPT and block some neuronal calcium channels.

The evidence suggests that both LPT and nifedipine act on a specific class or subtype of brain calcium channel, and may partially explain the discrepancies regarding the effects of dihydropyridines on neuronal calcium channels.

Supported, in part, by the Nelson Research and Development Company and the NIH.

231.12 MODULATION OF VOLTAGE-GATED CALCIUM CHANNEL FUNCTION BY ETHANOL. R.O.Messing*, C.L.Carpenter*, and D.A.Greenberg. Department of Neurology, University of California, San Francisco, CA 94110.

The activity of voltage-gated Ca channels is modified by a variety of drugs and ions, but little is known about long-term modulatory influences on channel function. Chronic exposure to ethanol alters Ca-dependent neurotransmitter release, which may be due to changes in depolarization-induced $Ca^{2+}$ entry into nerve terminals. To determine if the actions of ethanol involve changes in the function of voltage-gated Ca channels, we studied the effects of acute and chronic exposure to ethanol on 50 mM K-stimulated $^{45}Ca$ uptake and [$^{3}$H] nitrendipine ([$^{3}$H] NIT) binding in PC12 pheochromocytoma cells. In acute experiments, $^{45}Ca$ uptake was inhibited half-maximally at 205 mM ethanol and completely at 600 mM. For a series of n-alkanols, the relationship between uptake inhibition and alcohol chain length was linear, implying that inhibition of $^{45}Ca$ uptake is not due to increased osmolality alone. Cells cultured in 200 mM ethanol for 6 days demonstrated a 70-100% increase in K-stimulated $^{45}Ca$ uptake compared to control cells. This increase was not accompanied by a change in the acute response of Ca channels to ethanol, since $^{45}Ca$ uptake was inhibited to a similar extent by ethanol in chronically treated ($IC_{50}$ = 168 mM) and control ($IC_{50}$ = 178 mM) cells. To further investigate the mechanism underlying the increase in $^{45}Ca$ uptake observed in chronically-treated cells, we measured the number of channels present in membranes of cells treated with 200 mM ethanol for 6 days using [$^{3}$H] NIT as a channel-counting probe. Scatchard analysis gave $K_d$ values of 125 and 104 pM and $B_{max}$ values of 28.7 and 12.2 fmol/mg of protein for the treated and control cells respectively. The observed increase in binding site number may reflect a corresponding increase in the number of functional Ca channels, and thus explain the augmented $^{45}Ca$ uptake in cells chronically treated with ethanol. These findings suggest that Ca-dependent neuronal processes may be subject to long-term regulation by alterations in channel number induced by drugs or endogenous factors.

## INVERTEBRATE LEARNING AND BEHAVIOR III

232.1 NEURAL CORRELATES OF HABITUATION AND SENSITIZATION IN THE LEECH. S.R. Lockery and W.B. Kristan, Jr. Dept. of Biol., Univ. of Ca., San Diego, La Jolla, CA 92093.

A sudden increase in light intensity triggers an abrupt longitudinal contraction ("shortening") in Hirudo. Repeated presentation of light reduces probability of shortening and increases latency i.e., produces habituation (Lockery et al., Behav. Neurosci., 99: 333, 1985). Electric shock increases the probability of shortening and decreases latency i.e., produces sensitization. To investigate cellular bases of these phenomena, we used a semi-intact preparation allowing simultaneous recording of behavior and neural activity.

Abdominal segments 2-7 were left intact and ganglia 8-10, with their roots, were exposed for recording. Light and shock were delivered to segment 3. Shortening was monitored by a tension transducer attached at segment 2. A light pulse (18 s) produced a transient increase in longitudinal tension with two components: a small "primary response" with a time to peak tension of < 1 s, and a larger "secondary response" with a time to peak of many seconds. Motor neurons controlling shortening were recorded extracellularly. These cells responded with impulse bursts corresponding to the two components of increased tension.

The semi-intact preparation showed habituation and sensitization analogous to that of the whole animal. Primary response peak tension declined 60% over ten trials (one trial every 100 s). For control animals exposed only to trials 1 and 10, the decline was significantly less (5%). A mild shock immediately after trial 10 increased response on the next trial to 194% of the trial 1 value. Animals not shocked showed significant spontaneous recovery after 45 min. Similar results were obtained for secondary responses.

Changes in the activity of motor neurons correlated well with changes in the primary response. The neural response to light (the increase in firing frequency over baseline during a 1 s light pulse) was measured over 20 trials (one trial every 5 s). The neural response diminished with successive stimulations in parallel with the decline in primary tension. In fact, with data normalized to trial 1, the two curves could be superimposed. Similarly good correlation was obtained for sensitization.

These results demonstrate that motor neuron activity closely predicts the strength of the shortening response. Furthermore, they indicate that the changes responsible for habituation and sensitization in Hirudo are presynaptic to the motorneurons.

232.2 CELLULAR MECHANISMS OF ASSOCIATIVE LEARNING IN HERMISSENDA: CONTRIBUTION OF LIGHT-ACTIVATED CONDUCTANCES. T. Crow. Department of Physiology and Center for Neuroscience, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

Positive phototactic behavior of Hermissenda is modified by conditioning when light (CS) is paired with stimulation of the gravity-detecting statocysts (Crow and Alkon, 1978). Associated with the conditioned modification of phototaxis are a number of cellular correlates found in the sensory neurons (type B photoreceptors) of the CS pathway (Crow and Alkon, 1980; Alkon, Lederhendler, and Shoukimas, 1982; Crow, 1982; Alkon, 1984; Crow 1985). Recently, it was reported that conditioning results in a significant decrease in the amplitude of the photoresponse measured after 5 min of continuous illumination (Crow, 1985). The reduction in the photoresponse can not be explained by the previously reported reduction in $I_A$ of conditioned animals since adding 10 mM 4-AP to the bath ASW resulted in an increase in photoresponse amplitude measured after 5 min of continuous light (mean increase = 31.2%, n=7, $t_6$=13.4; $P < .005$). However the reduction in the amplitude of the photoresponses of conditioned Hermissenda could be due to changes in other voltage-dependent currents and/or light-activated membrane currents. In the experiments reported here I investigated the role of light-dependent currents in the reduction of the photoresponse of conditioned Hermissenda with voltage clamp techniques.

The training and testing procedures were identical to those used previously to demonstrate associative learning in Hermissenda (Crow and Alkon, 1978, Crow, 1985). Axotomized type B photoreceptors from conditioned and random control preparations were first dark adapted and then voltage clamped at their dark adapted resting membrane potential. Inward current evoked by a 100 msec light flash was measured in normal ASW and ASW containing 30 uM TTX from a holding potential of -55 mV or from the dark adapted membrane potential. After the light flash the clamp was turned off and the B-photoreceptors were dark adapted and then illuminated for 5 min with white light attenuated-2.0 log units. After 5 min of continuous illumination the B-photoreceptors were again voltage clamped and inward current evoked by a series of 100 msec light flashes was measured and compared to the pre-light adapted light-evoked membrane current. Conditioning resulted in a significant relative decrease in light-evoked inward current measured after 5 min of continuous ilumination as compared to random controls ($t_7$=2.58; $P < .05$). These results suggest that alterations in light-activated conductances contribute to the decrease in the amplitude of the generator potential of conditioned Hermissenda, and are consistent with previous reports of modifications of light and $Ca^{++}$-dependent processes underlying adaptation in type B-photoreceptors.

232.3 LONG-LASTING STIMULATION-INDUCED ENHANCEMENT OF SYNAPTIC TRANSMISSION IN THE CRAYFISH LATERAL GIANT ESCAPE CIRCUIT. M.W. Miller* and F.B. Krasne. Dept. of Psychology, University of California, Los Angeles, CA 90024.

The sensory limb of the crayfish lateral giant (LG) escape circuit is known to exhibit a use-induced depression of synaptic transmission, which underlies habituation of the tailflip response to repeated stimuli (Krasne, 1969; Zucker, 1972). We now report that brief periods of repetitive stimulation at frequencies higher than those which cause depression can cause substantial and sustained enhancement of transmission in the pathway.

Two types of enhancements were noted following trains to the second roots of abdominal ganglia 2-5. These differed in their time course and in the input pattern with which they were elicited.

(1) High frequency (50 Hz) trains delivered for brief durations (2 sec) resulted in a posttetanic potentiation (PTP) which lasted for 5-10 minutes.

(2) Low frequency (4 Hz) trains applied for 10 seconds produced a long-term potentiation ("LTP") which could last for hours.

Application of trains to neighboring ganglia failed to produce enhancements in "test" ganglia, indicating that neither type of potentiation is produced by a widespread interganglionic system such as that underlying sensitization (Krasne and Glanzman, 1984).

The long-term potentiation exhibited "cooperativity" like that noted in hippocampal LTP (Bliss and Gardner-Medwin, 1971; McNaughton et al., 1978). The potentiation produced by trains in which stimuli were stronger (2X) than the test stimuli was greater than that produced by trains in which stimuli were equal to the test intensity. This indicates that the LTP is not simply the result of repetitive firing of the "test" population of fibers but is influenced by coactivity of additional input fibers in the same pathway. Experiments are in progress to determine whether this coactivity sensitive potentiation is, like hippocampal LTP, associative in the sense that trains of strong stimuli to one set of afferents will selectively enhance the effectiveness of other afferents that are stimulated concurrently.

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232.4 LONG-TERM FACILITATION OF THE MONOSYNAPTIC CONNECTION BETWEEN SENSORY NEURONS AND MOTOR NEURONS OF THE GILL-WITHDRAWAL REFLEX IN APLYSIA IN DISSOCIATED CELL CULTURE. P. G. Montarolo*, V. F. Castellucci, P. Goelet*, E. R. Kandel, and S. Schacher (SPON: European Neuroscience Association). H. Hughes Medical Institute and Ctr. for Neurobiol. & Behav., Columbia Univ., College of P&S, and NYS Psychiat. Instit., New York, NY 10032.

The gill-withdrawal reflex of Aplysia undergoes short-and long-term sensitization both of which are mediated by facilitation of the direct connection between sensory neurons and motoneurons of the reflex. Recently it has become possible to reconstitute the neuronal circuit mediating this reflex in cell culture (Rayport, S. and Schacher, S., Neurosci. Abst. 10:923, 1984). As in vivo, the synapse between the sensory neurons and gill motor neuron L7 undergoes short-term facilitation when treated with 5-HT, a transmitter which simulates the effects of sensitizing stimuli. In the intact animal, long-term sensitization can be produced by presenting four or more facilitating stimuli at regular intervals. We have used an analogous protocol in the in vitro system, using repeated brief exposures to 5-HT, to explore the cellular and molecular mechanisms of long-term facilitation.

We co-cultured LE mechanosensory cells with the gill motor neuron L7 for six days, at which point the synaptic connections between them had formed and reached a stable amplitude. The EPSPs were determined, and then five bath applications of 5-HT (2 uM, 5 min each) were presented at intervals of 15 min. When retested 24 hrs later, the change in EPSP amplitude in the 5-HT treated cultures (+75 $\pm$ 31%, S.E.; n=6, 10 synapses) was significantly greater ($p<.01$) than the change in EPSP amplitude in control untreated cultures (-12 $\pm$ 8%; n=5, 8 synapses). Similar facilitation also occurred in a separate set of experiments using longer 5-HT exposure periods.

To determine whether this long lasting facilitation is dependent on protein synthesis, we are now studying the effects of protein and RNA nthesis inhibitors. As a first step, we have used anisomycin (10 um) which blocks protein synthesis by more than 90% and has no detrimental effects on evoked LE-L7 EPSPs. Anisomycin was added to cultures 30 min before starting the 5-HT protocol and kept in the cultures until 1 hr following the last 5-HT application. This treatment blocked the 5-HT-induced long-term increase of EPSP amplitudes (-17 $\pm$ 25%; n=4, 6 synapses) without interferring with short-term facilitation, indicating that long- and short-term facilitation can be dissociated. We are examining whether these results are due to a specific inhibition of protein synthesis.

232.5 SITE-SPECIFIC SENSITIZATION IN APLYSIA, LTP, REGENERATIVE BURSTING, AND A POSSIBLE LINK IN THE EVOLUTION OF LEARNING. E.T. Walters, Dept. of Physiol. & Cell Biol., Univ. of Texas Med. Sch., Houston, Texas 77225.

Activity-dependent neuromodulation of sensory neurons in Aplysia has now been implicated as a mechanism for both classical conditioning (Walters & Byrne, 1983; Hawkins et al, 1983) and long-term synaptic potentiation (LTP - Walters & Byrne, 1985). In order to explore the long-term behavioral and cellular effects of procedures known to produce activity-dependent neuromodulation of the tail sensory neurons, I have applied a cutaneous "LTP" training procedure both to restricted sites and to extensive fields in the tail region of intact, freely moving animals and later examined changes in defensive responses and in electrophysiological properties of sensory and motor neurons. The "LTP" training procedure consisted of 10 high frequency trains of shock (either 60 Hz or 25 Hz), each 0.5 sec long, applied to one side of the tail at 5 sec intervals. This brief (45 sec duration) training activated sensory neurons and produced facilitation of their monosynaptic EPSPs to tail motor neurons ($p < .05$) similar to that seen with nerve stimulation LTP (Walters & Byrne, 1985). It also enhanced tail and siphon withdrawals elicited by weak stimulation of the training site (relative to untrained sites) for at least 4 days after training ($p < .025$). While part of this site-specific sensitization may be peripherally mediated, a substantial central contribution was indicated by the greater sensitization seen when the tail was connected to the CNS than when the CNS was disconnected ($p < .05$), and by cellular correlates of the site-specific sensitization observed in sensory neurons in the isolated CNS ($p < .05$). A novel cellular correlate of sensitization was an enhanced likelihood of evoking regenerative bursts (2 to 28 spikes, lasting up to 2 sec) in the sensory neurons of the trained field in response to a 10 msec test depolarization delivered 1 day after training ($p < .01$).

Some have speculated that sensitization was an evolutionary precursor of classical conditioning. Finding that activity-dependent modulation can be a mechanism for both site-specific sensitization and defensive classical conditioning suggests an extension of this hypothesis: activity-dependent modulation in nociceptive systems may have evolved first to encode specific memory (in the form of LTP and regenerative bursting responses) of the sites of tissue damage; in nociceptive cells with a wide dynamic range this mechanism could also encode temporal associations between innocuous signals to one field and damaging events to another - a capacity which would then allow classical conditioning. (Supported by grants MH 38726 from the NIMH and 84-11-104 from the Searle Scholars Program).

232.6 CHOLINERGIC SUPPRESSION: A POSTSYNAPTIC CORRELATE OF LONG-TERM ASSOCIATIVE LEARNING IN PLEUROBRANCHAEA. A. D. Morielli*, E. M. Matera*, M. P. Kovac*, K. J. McCormack* and W. J. Davis. The Thimann Laboratories and Long Marine Laboratories, University of California at Santa Cruz, Santa Cruz, California, 95064

Although the neurotransmitter acetylcholine (ACh) has long been thought to play a role in learning and memory in vertebrates, the evidence for its involvement has been indirect. We here report that after specimens of the marine gastropod mollusk Pleurobranchaea californica have been food avoidance conditioned, the interneurons which command the feeding behavior (the PCN neurons) show a strong suppression of their depolarizing response to ACh.

In order to determine the differential response of PCN cells in brains taken from trained (experimental) and untrained (naive and control) animals, ACh was added to a preparation chamber in which a brain was maintained in sea water at 11°C. A bath concentration of $10^{-4}$ M ACh produced a mean depolarization of 14.1 mv $\pm$ 2.0 (standard error) in the PCN cells of brains taken from naive animals and a mean depolarization of 12.8 mv $\pm$ 4.4 in PCN cells of control animals. In contrast, PCN cells in brains taken from animals trained to avoid a food stimulus showed a mean depolarization of 1.6 mv $\pm$ 0.9 when stimulated with $10^{-4}$ M ACh and were depolarized only when exposed to a ten-fold higher concentration.

Receptors to ACh appear to be located on the soma of the PCN cells. Intracellular recordings were made from 2 adjacent PCN cells, both of which showed similar sensitivity to ACh. ACh ($10^{-2}$ M) was pressure ejected from a micropipette onto the soma of one of the two impaled PCN cells. The cell directly under the ACh pipette became depolarized by 15 mv immediately upon ejection of ACh, while the PCN cell adjacent to it showed no response, suggesting that rather than stimulating cells presynaptic to the PCNs, the ACh has a direct effect on the membrane of the PCN cells themselves.

The PCN cells of untrained animals were depolarized by the muscarinic cholinergic agonist, acetyl-$\beta$-methylcholine, but not by the nicotinic agonist, nicotine. The ACh induced depolarization in untrained animals was blocked by bath application of $10^{-4}$ M atropine, a muscarinic receptor blocker or, in 2 out of 3 cases, $10^{-4}$ M hexamethonium, a nicotinic receptor blocker. The ACh induced depolarization appears to be sensitive to external calcium concentrations and is also blocked by the phospholipase $A_2$ inhibitor quinacrine. These data suggest that the ACh receptors located on the soma of the PCN cells are more similar to the muscarinic receptors than the nicotinic receptors identified in vertebrates and that after avoidance conditioning, the depolarizing response of the PCN cells to ACh stimulation is suppressed.

232.7 LEARNED CHANGES OF FEEDING BEHAVIOR TO EDIBLE AND INEDIBLE FOODS IN APLYSIA. A.J. Susswein, M. Schwarz* and E. Feldman*. Dept. of Life Sciences, Bar-Ilan University, Ramat Gan, Israel 52100.

Aplysia learn to modify their feeding behavior when the behavior is paired with reinforcing consequences. Successful and failed attempts to transfer food from the buccal cavity to the gut respectively act as positive and negative reinforcers. Behavioral change is specific to feeding in response foods of a particular taste and texture. Plasticity is specific to pairing, as shown by lack of behavioral change when reinforcement is explicitly unpaired with feeding responses to a specific food. Memory of the response change persists for at least a week.

We determined whether neural mechanisms underlying regulation of feeding by associative learning may be related to mechanisms responsible for other processes regulating feeding, such as satiation or response decrement to sustained lip stimulation. A number of parametric features of feeding behavior were measured, to determine how they are affected by learning and by other forms of plasticity. If different processes modulating feeding operate via a common mechanism, the various parameters should be affected in a similar manner; by contrast, if unique mechanisms underlie each process the behavioral effects should also be distinct. Parametric features of feeding examined were: 1) efficacy of a feeding response in producing entry of food into the buccal cavity; 2) time that food remains in the buccal cavity after it enters; 3) number of attempted swallows elicited by food within the buccal cavity; 4) time that Aplysia maintain responsiveness to food; 5) specficity of response change to a particular food; 6) memory of response change. On the basis of differences in effects upon these parameters, learned changes in feeding behavior can be distinguished from those due to other processes. The data suggest that different CNS mechanisms are responsible for different forms of behavioral plasticity affecting Aplysia feeding.

Bilateral section of the esophageal nerves innervating the gut specifically impairs learned changes in feeding behavior, but not behavioral change due to sustained lip stimulation. Unilateral nerve section, or section of either of the two major divisions of the esophageal nerve, does not impair learning. These data suggest that stimuli necessary for reinforcement travel in this nerve. Such stimuli inform the CNS as to whether or not an attempt to ingest food was successful. The nature of gut stimuli necessary for learning is currently being explored.

232.8 **OPERANT CONDITIONING OF HEAD-WAVING IN *APLYSIA CALIFORNICA*** D.G. Cook* & T.J. Carew (sponsor: C.F. Stevens) Dept. Psychology Yale University, New Haven, CT 06520

An unresolved question in Psychology is whether classical and operant conditioning are fundamentally different or share a common mechanism. This issue might be clarified by comparing them on a cellular level. *Aplysia* could be useful for this analysis since progress has already been made in understanding the cellular mechanisms of classical conditioning. This led us to ask whether *Aplysia* can exhibit operant conditioning as well.

The operant response we examined was head-waving. Animals were trained to modify this response, increasing its frequency on one side of the body, in order to terminate the presentation of aversive bright lights. All experiments consisted of a 5 min baseline, 10 min of training, and a 5 min (non-reinforced) test. In the first experiment, animals receiving contingent reinforcement (N=10) spent significantly more test time on the non-reinforced ("safe") side compared to baseline ($p<.01$) and compared to yoked controls (N=10, $p<.05$). When the contingencies of reinforcement were reversed (safe side now punished and vice versa) the contingent group showed a marked decrease in responding on the now-punished side, and when the original contingencies were reinstated, they again increased their responding on the safe side. There was a significant trend ($p<.05$) in the contingent group's responding to these reinforcement contingencies, while yoked controls showed no significant trend. Two weeks later both groups of animals were retrained with contingent reinforcement. Both groups now showed significant conditioning ($p<.01$, $p<.05$, respectively) indicating that the previously yoked group was capable of conditioning if exposed to the appropriate reinforcement contingencies. In a second experiment we found that the learning was relatively resistant to extinction. Animals (N=10) were first given contingent training and showed significant learning ($p<.01$). They were the given 10 min of non-reinforced (extinction) training and still exhibited significant retention ($p<.005$). Only following reversed contingency training did they reduce responding on the safe side. In a final experiment we examined the cues used as feedback to learn the operant. To distinguish between visual and internally derived cues (eg. proprioceptive or efference copy) we trained as before but disrupted visual cues by rotating animals 180° before testing. The contingent group (N=10) still showed significant learning ($p<.006$); yoked controls (N=10) once again did not learn. Thus it appears that internal cues are used to code the operant response.

Neurons in both the reinforcement (Jacklet, 1980) and operant pathways (Henning, et al 1979) have previously been identified. Thus it is now possible to begin to analyze the cellular mechanisms of operant conditioning in *Aplysia*.

232.9 CONTEXT CONDITIONING IN APLYSIA CALIFORNICA. R. M. Colwill* (SPON: V. Castellucci). Howard Hughes Medical Institute for Molecular Neurobiology, Center for Neurobiology & Behavior, Columbia University, College of P&S, and N.Y.S. Psychiatric Institute, New York, NY 10032.

Studies of the associative capacities of invertebrates have focussed on learning yielded by pairings of conditioned (CS) and unconditioned stimuli (US). Less attention, however, has been paid to learning about the context in which those pairings occur. That neglect is surprising given the important role that informational theories of Pavlovian conditioning assign to contextual stimuli. Those theories assume that several complex learning phenomena (e.g., sensitivity to CS-US contingency) depend on the development of associations between contextual stimuli and USs. For that reason, it is important to demonstrate contextual learning in preparations used for neurophysiological analyses of learning. This paper examines context-US associations in Aplysia whose nervous system has been studied in detail.

Subjects received shock USs in one context but not in another. The success of that treatment in conditioning the context was evaluated in two ways; first, by the impact of the context on responding, and second, by its ability to interfere with learning. Sixteen Aplysia were exposed to two different contexts: a smooth round bowl containing lemon flavored sea-water and a lined rectangular box containing plain sea-water and an aerator. Several moderate electric shocks were delivered to the mantle shelf in one context but not in the other. To assess context conditioning, we first measured the defensive reaction of each subject in the presence of the contextual cues. Subjects exhibited more fear in the shocked context than in the nonshocked context as indexed by the duration of siphon withdrawal in response to mild siphon stimulation ($p< .05$). The second assessment of contextual conditioning exploited a finding that has been well documented in vertebrates: pairings of a CS and US are less effective when delivered in a context that has previously been associated with that US. Consequently, we examined the ability of the shocked context to interfere with conditioning of a CS that was paired with the mantle shock reinforcer. All subjects received 8 pairings of mild siphon stimulation with mantle shelf shock. For half the animals, that training occurred in the shocked context; for the other half, it occurred in the nonshocked context. All subjects were then given a nonreinforced presentation of the siphon stimulus in a third context (home tank). The duration of siphon withdrawal elicited by the CS was longer if conditioning had taken place in the nonshocked context than in the shocked context ($p<.05$). That outcome suggests that the initial context-shock pairings established a context-US association. These results suggest that Aplysia californica can form an association between a shock US and the context in which that US occurs. That association was revealed both by enhanced defensive reactions in the shock-associated context and by the ability of that context to interfere with conditioning of a discrete CS paired with that shock. To our knowledge, this provides the first demonstration of blocking in Aplysia. That result is of substantial significance because of the central role blocking is accorded by informational theories of Pavlovian conditioning.

232.10 OPERANT CONDITIONING AND DIFFERENTIAL CLASSICAL CONDITIONING OF GILL WITHDRAWAL IN APLYSIA. R. D. Hawkins, G. A. Clark, and E. R. Kandel. Howard Hughes Medical Institute, Center for Neurobiology & Behavior, Columbia University, and N. Y. State Psychiatric Institute, New York, NY 10032.

Associative learning has traditionally been divided into two major categories: classical and operant conditioning. To determine how these two forms are related, it would be desirable to examine them in the same simple behavioral system. Classical conditioning has been demonstrated for gill and siphon withdrawal in Aplysia (Carew et al., 1981), and operant conditioning has been demonstrated for head waving (Cook & Carew, 1985). The cellular mechanism of classical conditioning of the withdrawal response has been analyzed (Hawkins et al., 1983). We now report that the gill component of the withdrawal response, which undergoes differential classical conditioning, also undergoes operant conditioning.

Animals were restrained with hooks attached to the parapodia and a movement transducer was attached to the gill. To study differential classical conditioning, two CS electrodes were clipped to the siphon to deliver the conditioned stimuli (CSs) and a pair of electrodes was implanted in the tail to deliver the unconditioned stimulus (US). During training, stimulation of one CS electrode was paired with and preceded the US by 0.5 seconds, while stimulation of the other CS electrode was specifically unpaired with the US. Fifteen to sixty minutes after 5 training trials, the amplitude of the response to the paired CS was significantly greater than that of the response to the unpaired CS (73% vs. 53% of pretest in 20 experiments, $p<.05$), demonstrating differential classical conditioning.

To study operant conditioning, the same preparation was used except that the mantle shelf was glued to a fixed rod to minimize body movement, and a single electrode was implanted in the siphon. Animals were run in pairs. During the first phase of the experiment, one animal (the contingent animal) received siphon shock each time its gill relaxed beyond a criterion level (approximately 20% of full contraction) and the other animal (the yoked control) received shock at the same time, regardless of its gill position. During the second phase of the experiment, shock was contingent on gill position for both animals. Each phase consisted of two 10 minute training periods alternating with 10 minute extinction periods. Animals which received contingent training in phase I spent a significantly greater percent of the time with their gills contracted beyond the criterion level than did control animals in all segments of the experiment: that is, during training (91% vs. 64% in 20 pairs of animals, $p<.01$) and extinction (38% vs. 22%, $p<.05$) in phase I and also during training (82% vs. 73%, $p<.05$, 1-tail) and extinction (34% vs. 20%, $p<.05$) in phase II, demonstrating operant conditioning.

Since much is already known about the neural control of gill withdrawal, it may now be possible to study the mechanism of operant conditioning as well as the relationship between operant and classical conditioning of this response on the cellular level.

232.11 ACTIVATION OF ADENYLATE CYCLASE IN APLYSIA NEURAL TISSUE BY $Ca^{2+}$/CALMODULIN, A CANDIDATE FOR AN ASSOCIATIVE MECHANISM DURING CONDITIONING. T. W. Abrams, L. Eliot, Y. Dudai and E. R. Kandel. Center for Neurobiology & Behavior, and Howard Hughes Medical Institute, Columbia University, College of P & S, N. Y. State Psychiatric Institute, New York, NY 10032.

In classical conditioning of the siphon withdrawal reflex of Aplysia, the reflex response to the conditioned stimulus, a siphon touch, is enhanced if the CS is presented immediately prior to the unconditioned stimulus (US), a tail shock. On the cellular level, this temporal specificity may be substantially accounted for by the fact that spike activity in the siphon sensory neurons enhances their response to the presynaptic facilitatory input resulting from the US. Thus facilitation of the synaptic connection from the sensory neurons that produce the reflex occurs optimally when action potentials in the sensory neurons have been triggered by the CS just before the presentation of the US (Hawkins et al., 1983). Moreover, our evidence suggests that the $Ca^{2+}$ influx that occurs during this paired spike activity is critical for the enhancement of the facilitation response (Abrams et al., 1983).

We have now begun to analyze the basis of this temporal specificity on the molecular level. Because paired spike activity and the resulting $Ca^{2+}$ influx enhance the elevation of cAMP that mediates presynaptic facilitation in the sensory neurons (Abrams et al., 1984), we have investigated whether the adenylate cyclase in Aplysia nerve cells is modulated by $Ca^{2+}$/calmodulin. Three lines of evidence suggest that the adenylate cyclase in particulate membrane from Aplysia neurons is indeed activated by calmodulin, as is the cyclase in mammalian brain. 1) Stimulation of the cyclase by $Ca^{2+}$ plus 5-HT is substantially reduced by the calmodulin inhibitor R24571 (84 $\pm$ 21% stimulation reduced to 18 $\pm$ 14% stimulation) or by a second inhibitor, W7. 2) After membranes are depleted of endogenous calmodulin by washing first with trifluoperazine and then with EGTA, there is little cyclase stimulation by either $Ca^{2+}$ or 5-HT; in contrast, after addition of mammalian brain calmodulin, $Ca^{2+}$ in combination with 5-HT produces more than two fold activation of the cyclase. 3) Approximately 25% of the adenylate cyclase activity from solubilized Aplysia nervous tissue membrane binds to calmodulin-Sepharose in a $Ca^{2+}$-dependent manner; eluting this activity with EGTA yields cyclase that is stimulated 2-fold by mammalian calmodulin, but is not stimulated by regulatory G protein activators, suggesting that calmodulin binds directly to the catalytic unit. We are presently using the membrane homogenate to investigate whether the dual activation of the calmodulin-dependent cyclase can account for the temporal specificity observed in the conditioning of the reflex.

232.12 ABSENCE OF STIMULATOR-INDUCED AFFINITY SHIFTS IN AN ADENYLATE CYCLASE SYSTEM FROM THE DROSOPHILA MEMORY MUTANT RUTABAGA. Yadin Dudai, The Weizmann Institute of Science, Rehovot 76100, Israel.

The Drosophila mutant rutabaga (rut) fails to remember normally and is defective in a subpopulation (or in a functional state) of adenylate cyclase. We report here that the mutation leaves some tissues in rut with a membrane-associated adenylate cyclase that cannot normally switch between different states of affinity for its ligands. The results described below were obtained with abdominal preparations, in which the rut cyclase defect is especially (but not exclusively) pronounced. The enzyme in normal flies displays a higher affinity for MgATP and a lower affinity for free $Mg^{2+}$ than the residual enzyme in the same rut tissues. The diterpene forskolin markedly increases the Vmax of Drosophila adenylate cyclase and this stimulation is accompanied by a downward shift in the affinity for MgATP (apparent Km is increased ca. 3-fold) and by an upward shift in the affinity for free $Mg^{2+}$ (the apparent Km is decreased ca. 3-fold). In contrast, no shifts in the affinity for MgATP and for free $Mg^{2+}$ are observed in the rut enzyme. The dose-dependence of the forskolin stimulation in rut also differs from normal. When adenylate cyclase is solubilized by Lubrol-PX to a form that is not stimulated by guanyl nucleotides, the rut enzyme displays lower affinity for MnATP than the normal solubilized enzyme. In such a solubilized preparation there are no significant differences between the activation of the normal and the mutant enzyme by free $Mg^{2+}$ and by free $Mn^{2+}$. A striking difference is however observed in the mode of activation of the Lubrol-solubilized adenylate cyclase by forskolin: the normal enzyme is markedly activated by the diterpene whereas the rut enzyme shows only little activation. Taken together with previous results, the data indicate that adenylate cyclase in rut differs from the normal enzyme in several properties (i.e., low Vmax and low affinity for MgATP, defective interaction with $Mg^{2+}$ and lack of stimulation by low $Ca^{2+}$ concentrations). The defects in rut thus seem to be associated both with the catalytic unit and with its mode of interaction with N units. These defects may be due either to a mutation in a gene that codes for a catalytic subunit or in a gene that codes for a yet unidentified regulator which is tightly associated with the catalytic unit. Sustained activation of adenylate cyclase has been suggested to underlie short-term memory in Aplysia (Castellucci et al., Soc. Neurosci. Abst. 9, 169 (1983)). According to such a model, defective stimulation of adenylate cyclase and inability to switch properly between activity states (e.g., due to malfunctioning of "Mg-switches" in the cyclase system, see Iyengar and Birnbaumer, PNAS 79, 5779 (1982)) should result in abortive memory. It is thus possible that lack of the capacity of an adenylate cyclase system to appropriately shift between activity states results in defects in behavioral plasticity. (Supported by the The Fund for Basic Research, The Israel Academy of Science, Jerusalem).

## MOLECULAR BIOLOGY OF GENE EXPRESSION AND NUCLEIC ACIDS III

233.1

WITHDRAWN

233.2 COMPLETE NUCLEOTIDE AND DEDUCED POLYPEPTIDE SEQUENCES OF BOVINE PHENYLETHANOLAMINE N-METHYLTRANSFERASE. E.E.Baetge, Y.H. Suh and T.H. Joh. Dept. of Neurology, Cornell Univ. Med. Coll., New York, NY 10021,

Phenylethanolamine N-methyltransferase (PNMT) (E.C.2.1.2.28, S-adenosyl-L methionine, phenylethanolamine N-methyltransferase), is the final enzyme in the catecholamine biosynthetic pathway. The enzyme is essential for both the biosynthesis of the hormone epinephrine in the adrenal medulla as well as the neurotransmitter epinephrine in the brain. In an effort to better understand how the expression of the enzyme may be regulated at the molecular level and to further elucidate the molecular structure of the PNMT gene, we have recently isolated a cDNA clone to bovine PNMT (Baetge et al., Neurochem. Intl. 5(no.5):611-617, 1983.

Using this partial length clone, we have screened a lambda gt11 recombinant DNA expression library constructed using bovine adrenal medulla poly(A)+ mRNA as template. A cDNA clone containing the full coding region of PNMT was isolated. The cDNA clone is 1 kb in length and includes 40 bp of 5' and 180 bp of 3' untranslated sequence.

The complete nucleotide sequence of the cDNA was determined by the method of Maxam and Gilbert (1977). We report here the entire primary structure of PNMT as deduced from this nucleotide sequence.

233.3 RATES OF PHENYLETHANOLAMINE N-METHYLTRANSFERASE GENE TRANSCRIPTION DIFFER IN FISCHER AND BUFFALO RATS. M. Evinger, E.E. Baetge and T.H. Joh. Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

Production of the epinephrine synthesizing enzyme phenylethanolamine N-methyltransferase (PNMT, E.C. 2.1.1.28) differs in two inbred rat strains, Buffalo and Fischer 344. Observations that the Fischer strain exhibits higher levels of epinephrine synthesis and PNMT enzymatic activity have previously been extended by the demonstration that the amounts of immunotitratable PNMT protein and hybridizable PNMT mRNA are greater in the Fischer rats. Quantitative dot blot hybridizations have confirmed that the steady state level of PNMT mRNA in the Buffalo strain is lower both in the adrenal gland, the major site of synthesis, and in PNMT producing regions of the CNS, specifically in the medulla oblongata.

This study analyzes the synthesis of PNMT mRNA in order to determine whether the strain specific differences in PNMT expression are mediated at the level of gene transcription. Transcription of PNMT mRNA has been compared in the cellular nuclei of adrenals isolated from adult male Fischer and Buffalo rats. An in vitro nuclear transcription assay was employed to measure the rate of PNMT mRNA synthesis as a function of the amount of hybridization of $^{32}$P-labelled adrenal mRNAs to a PNMT-specific cDNA (710 bp). The rates of adrenal PNMT gene transcription differ significantly in the Buffalo and Fischer rats. Reflecting the relative levels of PNMT protein and mRNA, transcription of the PNMT gene proceeded at a rat 2-6-fold greater in the Fischer strain. Furthermore, consistent with its intermediate level of enzymatic activity, PNMT mRNA in Sprague-Dawley rats was transcribed at a ratio of 2 : 1 : 0.3 in a comparison of Fischer : Sprague Dawley: and Buffalo rats. Northern blot analysis indicates that PNMT mRNA is the same size in all these strains. Therefore, the rate at which the PNMT gene is transcribed must constitute a major determinant in the strain specificity of PNMT expression in the rat adrenal. Measurement of PNMT mRNA synthesis in its other sites of synthesis will reveal whether an inherent strain specific capacity governs the level of transcription possible for PNMT gene expression. (Supported by NIH Grants RR05396, HD19427, and MH24285.)

233.4 RECOMBINANT DNA PROBES FOR GENES EXPRESSED IN THE CEREBELLUM. T.L. Wood* and A.J. Tobin (SPON: J.S. de Vellis). Dept. of Biology and Molecular Biology Institute, Univ. of California, Los Angeles, CA 90024.

We are investigating gene expression in the cerebellum of humans and mice. By differential screening of a human cerebellar cDNA library in pBR322 we have found several RNA species that appear to be preferentially expressed in the cerebellum.

We are using RNA blot hybridization to investigate the regional specificity of the expression of these sequences. Preliminary evidence with this more sensitive technique indicates that some of the sequences previously identified are cerebellum-specific; others appear in the cerebellum and cerebral cortex but not in the neostriatum or liver; still others are found in all three brain regions, but not in liver and kidney. We are currently examining several neuronal and glial cell lines in order to determine whether any of these sequences are neuronal- or glial-cell specific.

We have recently constructed a λgt11 expression cDNA library from adult (3-4 week) mouse cerebella. This library consists of more than 2 x $10^6$ recombinants. We are screening this library for several specific genes of interest using antibodies against cerebellar proteins.

These experiments will allow us to study the regulation of individual genes of both known and unknown functions during the development of the cerebellum.

This work is supported by grants to AJT from the Dystonia Medical Research Foundation and the NIH (#NS20356 and NS22256). TLW is supported by USPHS Training Grant #GM-07191 in Integrative Biology.

233.5 CEREBELLUM-SPECIFIC GENES OF THE MOUSE. W.Wille, UAO. Heinlein*, C.Ruppert*, and H.Schaal*. Institute for Genetics, University of Köln, D-5 Köln 41, West-Germany

Three region- and age-specific cDNA libraries from cerebellum and cerebral cortex of the mouse have been constructed (Wille, W. et al., Soc. Neurosci. Abstr. Part 1, 10:374, 1984). Using a three-step procedure our cDNA library from adult cerebellum was screened for sequences expressed in the adult cerebellum exclusively.

First, 3000 randomly picked (out of 250,000) clones were grown as glycerol stocks in microtiter plates and transferred to Whatman 541 filters. The DNA has been prepared on the filters according to Taub, F. and Thompson, E.B. (Anal. Biochem., 126:222, 1982). Identical filters were hybridized with single stranded cDNA from adult mouse cerebellum (CbA-cDNA) and adult total brain minus cerebellum (TBmA-cDNA), respectively. We identified 26 clones which hybridized with CbA-cDNA only.

Second, the plasmids of all 26 clones were digested with BamHI (or BamHI/HindIII) in order to cut out the cDNA inserts. The restriction fragments were separated by agarose gel chromatography, blotted on nitrocellulose filters, and hybridized ('Southern-blot') again with CbA- and TBmA-cDNA, respectively. At least 10 cloned cDNA inserts only hybridized with CbA-cDNA.

Third, the positive clones from the second screening step were hybridized ('Northern-blot') against mouse mRNAs of different origin: poly(A)+mRNAs from adult cerebellum, neonatal (postnatal day 1-2) cerebellum, adult total brain minus cerebellum, neonatal total brain minus cerebellum, adult kidney, adult liver and poly(A)-mRNA from adult cerebellum. For at least 4 of the clones a positive signal on 'Northern-blots' has been observed only with mRNA from adult cerebellum. These clones are named "CebA-#".

The sequence on one of the clones, CebA-117, shows striking homology to portions of the mouse kallikrein gene. For the time being, it is not clear whether CebA-117 is a member of the kallikrein gene family.

Detailed data on the genomic organization, sequence, localization and developmental regulation of cerebellum-specific clones will be presented.

This work was supported by the grants Wi563/3-2 and Wi563/3-3 of the Deutsche Forschungsgemeinschaft (SPP "Biochemie des Nervensystems") to W. Wille.

233.6 MICROINJECTION OF RNA INTO XENOPUS OOCYTES AS A TOOL FOR MOLECULAR CLONING OF A RAT BRAIN SEROTONIN RECEPTOR GENE. H. Lübbert*, N. Dascal*, T.P. Snutch*, H.A. Lester and N. Davidson* (SPON: A.M. Gurney). Divisions of Chemistry and Biology, Caltech, Pasadena, CA 91125.

The low abundance of most voltage and/or neurotransmitter sensitive receptors and ion channels renders it difficult to prepare pure proteins and obtain either amino acid sequence data or antibodies. Since for nearly all channels and receptors these useful tools for molecular cloning are unavailable, we envisioned a cloning strategy based on the observation of Barnard et al. (1982, Proc. R. Soc. Lond. B **215**, 241-246, and later papers) that a number of voltage-sensitive or neurotransmitter-responsive channels appear on the surface membrane of Xenopus oocytes after microinjection of rat brain poly(A)$^+$ RNA.

We propose to use this as a selection system for the cloning of a rat brain serotonin (5-HT) receptor gene. This approach has also aided in the cloning of other genes for which activity tests are applicable. The oocyte membrane contains an endogenous muscarinic acetylcholine (ACh) receptor which is coupled to a chloride channel (Kusano et al., 1977, Nature **270**, 739-741; Dascal et al., 1984, J. Physiol. **352**, 551-574). It has been shown that after injection of rat brain poly(A) RNA into Xenopus oocytes a rat brain 5-HT receptor is also coupled to a chloride channel (Gundersen et al., 1983, Proc. R. Soc. Lond. B **219**, 103-105). The waveform of the response to 5-HT resembles the D1 and D2 components of the muscarinic response of the oocyte membrane. A response to 5-HT inhibits a subsequent response to ACh and vice versa. Similar inhibition is observed if either ACh or 5-HT is applied twice to an oocyte within a few minutes. We suggest that a rat brain 5-HT receptor and the endogenous ACh receptor are coupled to the same chloride channels, perhaps via the same second messenger system.

The first step in our intended cloning procedure was to size fractionate rat brain poly(A)$^+$ RNA by electrophoretic separation through agarose gels. A single ~6 kb RNA fraction, enriched at least 60-fold, was sufficient to induce a serotonin response in the oocytes. The response to serotonin still included the D1 and D2 components. We have constructed cDNA clones of this ~6 kb RNA fraction and are working on a selection procedure for positive clones.

Supported by GM-10991, NS-11756, Deutsche Forschungsgemeinschaft, Bantrell Foundation and Fulbright Award.

233.7 EXPRESSION OF HIGH AFFINITY, GABA UPTAKE SYSTEM IN XENOPUS OOCYTES MICROINJECTED WITH BRAIN POLY $A^+$-RNA. P. Vijay Sarthy and Brian Hilbush*. Dept of Ophthalmology, University of Washington School of Medicine, Seattle WA 98195.

We are interested in the isolation and characterization of mRNAs encoding neurotransmitter uptake systems. We have used Xenopus Oocytes, microinjected with brain poly $A^+$-RNA, as the expression system to screen for the mRNAs encoding the polypeptide/s involved in GABA uptake. Poly$A^+$-RNA was isolated from developing rat brain and microinjected into mature Oocytes by established techniques. Injected Oocytes were left overnight in modified Barth solution before use. The controls consisted of Oocytes injected with Barth's solution instead of RNA. For measurement of GABA uptake, 8-10 injected Oocytes were pooled and incubated for 10 min with μmolar concentrations of $^3$H-GABA. After brief rinses, radioactivity accumulated was determined by scintillation counting.

In Oocytes injected with RNA, $^3$H-GABA accumulation was about 3-fold higher than in Barth-injected controls. When uptake was measured at 18°, 30° or 37°C, maximal levels of uptake were found in Oocytes incubated at 30°C. At this temperature, $^3$H-GABA uptake continued to increase up to 20 min and declined thereafter. In order to determine the kinetic parameters of uptake, RNA-injected Oocytes were incubated with varying concentrations of $^3$H-GABA (0.1-25 μM) and the $^3$H-radioactivity accumulated was determined. It was found that GABA uptake could be described by a single, high affinity system with a $K_m$ = 7 μm and a $V_{max}$ of 0.12 pmoles/min/mgp. $^3$H-GABA uptake was blocked by DABA (87.4%), β-alanine (92.3%), and Nipecotic acid (72.6%). Furthermore, in $Na^+$-free medium, GABA uptake was reduced to 90.5% of the control. These results establish that Xenopus Oocytes, microinjected with poly $A^+$-RNA from developing brain, express a $Na^+$-dependent, high affinity GABA uptake system.

Supported by NIH Grants EY-03523, -03664, and -01730.

233.8 EXPRESSION OF RAT TUBULIN MULTIGENE FAMILY DURING BRAIN DEVELOPMENT. I. Ginzburg and U.Z. Littauer. Dept. of Neurobiology, Weizmann Institute of Science, Rehovot, Israel.

The diversity of microtubule functions and the temporal employment of microtubules through cell growth and differentiation suggests that the expression of tubulin genes may be tightly regulated. Microtubules are particularly important in the brain where they are involved in cell differentiation and synaptic transmission. In mammalian DNA, hybridization experiments with tubulin cDNA probes have revealed the presence of α-and β-tubulin multigene families. However the number of functional genes is unknown. To study the expression of an individual member of the tubulin gene family, specific cDNA probes were constructed.

Two α-cDNA clones were isolated and sequenced. These two clones share high homology within the coding region, however the 3'-non-coding regions are highly divergent. A strong interspecies homology exists in the 3'-non-coding region when compared with α-tubulin sequences from other mammals i.e. hamster and human cells. The interspecies homology suggests functional requirement for that region. Both α-tubulin genes encode for an identical size mRNA of 1.8-k.b.

The nucleotide sequence of a rat brain β-tubulin has been determined. The coding region shows a high homology when compared to chicken and human β-tubulin sequences. However the C-terminal coding end shows high divergence and no homology is observed at the 3'-non-coding regions. Three β-tubulin mRNA species are present in rat brain. A dominant neuronal 1.8-k.b. species and two minor species of 2.6 and 2.9-k.b. respectively. The 2.9-k.b. and 2.6-k.b. mRNAs represent an early and a late neuronal species respectively. By using oligonucleotide probes we have demonstrated that the three mRNAs are distinct species, which are developmentally regulated.

These data illustrate that the differential expression of the tubulin multigene family during rat brain development, which may suggest for different functions of the various tubulin isotypes. The specific probes and synthetic oligonucleotides are used for in situ hybridization studies of rat cerebellum which show differential tubulin mRNA levels in identified cell types.

Acknowledgement
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233.9 EXPRESSION OF CHICKEN ACTIN GENES DURING MYOGENESIS. L. J. Hayward*[1] and R. J. Schwartz*[1,2]. (SPON: Y. Clement-Cormier). [1]Program in Neuroscience and [2]Dept. of Cell Biology, Baylor College of Medicine, Houston, Texas 77030.

Embryonic muscle development has provided a cell differentiation system to study the regulation of contractile protein genes. Studies in the intact embryo have shown that chemical denervation with d-tubocurarine inhibits the normal appearance of secondary myotubes. However, in the absence of functional innervation, myogenesis in vitro proceeds and follows a succession of morphological stages which include proliferation of myoblasts, fusion of mononucleated cells, and the appearance of myofibrils. We have recently characterized the chicken actin gene family and have shown that actin gene expression is switched during myogenesis. In order to distinguish the regulation of individual actin mRNA species, we have constructed hybridization probes from the transcribed 3'-noncoding region of the single-copy α-skeletal, α-cardiac and β-cytoplasmic actin genes. Fragments (about 200 bp long) 3' to the translational stop codon were isolated from pAC51 (α-Sk), pAC7.5 (α-Ca), and pAC1 (β-Cyt) actin clones, and subcloned into M13 vectors. Each probe hybridizes to a single genomic locus with no significant homology to either of the other two actin mRNA species.

These probes were used to quantitate the steady-state levels of actin mRNAs during embryonic thigh development. α-cardiac and β actin mRNAs composed 48% and 38%, respectively, of the actin transcripts at day 10 in ovo, while α-skeletal actin mRNA made up only 14% of the total actin messages. However, by 17 days in ovo the α-skeletal mRNA had increased over 30-fold and represented over 90% of the total actin mRNA. In contrast, α-cardiac mRNA decreased by 30% from the level found at day 10, and β actin mRNA was reduced by 70%. The expression of actin genes was then examined in primary myoblast cultures. By 36 h in culture, α-cardiac mRNA had increased to levels about 10-fold higher than the α-skeletal message. The α-skeletal expression remained low until 48 h (after fusion had begun), suggesting that these two actin genes are influenced by separate control mechanisms. β actin mRNA levels increased sharply during the proliferative phase prior to fusion but peaked at 36 h and steadily declined thereafter. At the completion of fusion, the α-skeletal mRNA had increased 10- to 30-fold over the initial level in proliferating myoblasts. The α-cardiac expression remained high (40% of total) throughout post-fusion myogenesis. We conclude that primary myoblast cultures partially mimic the in vivo embryonic state. Full induction of α-skeletal actin mRNA and down-regulation of α-cardiac mRNA in the intact embryo may require neural influences not present in culture. The role of innervation upon actin gene expression in myogenesis is currently being investigated.

233.10 CRF INCREASES ACTH SECRETION AND POMC mRNA LEVELS IN ANTERIOR PITUITARY CELLS THROUGH AN ACTIVATION OF cAMP DEPENDENT PROTEIN KINASE. T. Reisine, G. Rougon*, J. Barbet* and H. Affolter*. Lab. of Cell Biology, National Institute of Mental Health and Lab. Math. Biology, National Cancer Institute, Bethesda, MD 20205.

Corticotropin releasing factor (CRF) is the most potent and effective natural stimulant of adrenocorticotropin (ACTH) release. In a tumor cell line of the mouse anterior pituitary, AtT-20/D16-16, CRF also increases the levels of proopiomelanocortin (POMC) mRNA. The levels of a larger sized nuclear RNA species with the expected size of the primary transcript for the POMC gene was also enhanced by CRF suggesting that the peptide activates the POMC gene. 8-Bromo-cAMP stimulates ACTH release and elevates the levels of POMC mRNA in AtT-20 cells and CRF activates cAMP dependent protein kinase in these tumors indicating that the effects of CRF on ACTH secretion and synthesis may be mediated through cAMP dependent protein kinase. To test this possibility, the inhibitor protein of cAMP dependent protein kinase (PKI) was incorporated into AtT-20 cells. This was accomplished by encapsulating the PKI into liposomes which were then coupled to protein A. The liposomes were then applied to AtT-20 cells prelabeled with anti-NCAM antibodies. These antibodies bind to AtT-20 cells and can be internalized. The protein A-bearing liposomes containing the PKI attached to the antibodies bound to the cells and were subsequently endocytosed. This resulted in the delivery of some of the encapsulated PKI into the cell cytoplasm as demonstrated by the blockade of 8-bromo-cAMP-, forskolin- and CRF-stimulated ACTH release but not of hormone secretion induced by $K^+$ or phorbol esters. The PKI also prevents CRF and 8-bromo-cAMP but not phorbol ester from elevating POMC mRNA levels in AtT-20 cells, indicating that CRF acts through cAMP-dependent protein kinase to stimulate ACTH secretion and the POMC gene. The PKI by itself also lowers POMC mRNA levels further suggesting a regulation of the gene by cAMP. The ability of phorbol esters to stimulate both ACTH release and synthesis implies that both responses are controlled by at least two distinct protein kinases.

233.11 CHOLECYSTOKININ mRNA AND PEPTIDE IN RODENT BRAIN: REGIONAL LOCALIZATION AND ONTOGENY. M. J. Iadarola, T. T. Quach*, A. M. Duchemin* and E. Costa. Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hospital, Washington, D.C. 20032.

The regional location of CCK biosynthesis and storage in rat and mouse brain was studied using specific cDNA hybridization and radioimmunoassay techniques to measure CCK mRNA and CCK immunoreactivity, respectively. The pre- and post-natal development of CCK gene expression and CCK peptide formation was also examined. For the regional distribution, the brain was grossly dissected into seven regions: cerebral cortex, hippocampus, striatum, hypothalamus, midbrain, cerebellum and pons-medulla. Radioimmunoassay was performed with an antibody directed to the C-terminal amide of CCK-8 after extraction of tissue peptides with 90% methanol. In rat brain, rank order (highest to lowest) for tissue content of CCK immunoreactivity (on a per mg protein basis) was cerebral cortex, hippocampus, caudate midbrain, hypothalamus pons-medulla cerebellum.

CCK mRNA was measured by hybridization to a cDNA probe obtained from plasmid pCK2AB5 (Proc. Natl. Acad. Sci. 81: 726, 1984). A 147 base pair AVA I/XMA III restriction fragment was labeled with $^{32}$P-dCTP by nick translation for hybridization. After RNA extraction and enrichment for poly A$^+$ RNA by oligo dT cellulose chromatography, agarose gel electrophoresis was performed followed by transfer to nitrocellulose and hybridization to the cDNA probe. A RNA of approximately 800 nucleotides was observed and there was no obvious CCK mRNA size differences between brain regions. The rank order of regional CCK mRNA content was cortex, hippocampus, midbrain hypothalamus, striatum, brain stem, cerebellum. At the level of resolution afforded by the dissection, cortex, hippocampus and midbrain were the three major regions in which CCK synthesis was observed, all others were near background. Little or no CCK mRNA was observed in striatum despite a high amount of immunoreactive peptide suggesting that the CCK content of striatum was derived mainly from afferent terminals and not from intrinsic perikaryal synthesis. A similar regional profile of CCK mRNA was observed in mouse brain.

The ontogenesis of CCK-RNA and peptide closely paralleled one another. The major appearance of CCK-RNA occurred post-natally as did the peptide immunoreactivity. Peak production occurred between days 7-21.

The results provide a new perspective on the brain CCK system attainable with hybridization techniques. We have defined major sites of CCK synthesis and increased current knowledge on this system acquired mainly by immunocytochemistry and radioimmunoassay. Further studies are aimed at a more precise localization of CCK-RNA production in the midbrain and the use of RNA hybridization to assess the synthesis of utilization of this peptide in CCK-producing neurons.

233.12 TRANSFER HYBRIDIZATION: A NOVEL METHOD FOR mRNA ABUNDANCE DETERMINATION AND cDNA LIBRARY SCREENING. S.A. Stein*, B. Trus* and M. Zasloff* (Sponsor: L. Mercer), Dept. of Neurology, Univ. of Texas Southwestern Medical School, Dallas, Texas 75235 and Section of Human Genetics, Lab. of Molecular Biology, NIH, Bethesda, Md. 20205.

Transfer hybridization, a novel, sensitive (≤ 1 copy of mRNA/cell), and specific method for broad range mRNA abundance determination and cDNA library screening has been developed. A detailed description of the method will be presented using rat brain and rat liver cDNAs. Sensitivity, specificity, and comparative abundance data have been confirmed by solution and dot blot hybridization. Transfer hybridization alone and in combination with sequential colony hybridization on 541 Whatman filter sheets have been used for screening and characterization of rat cerebellum and rat liver cDNA libraries. Quantification and autoradiographic background subtraction on the transfer and colony hybridizations have been accomplished by densitometry with computerized image processing. These methods have also been applied to cDNAs reflective of specific mRNAs that are changed in the euthyroid to hyperthyroid transition.

## TRANSMITTER CYTOCHEMISTRY AND IMMUNOHISTOCHEMISTRY I

234.1 **IMMUNOGOLD LOCALIZATION OF *APLYSIA* NEUROPEPTIDES** T. Kreiner and R.H. Scheller, Dept. of Biological Sciences, Stanford, CA 94305-2493.

Many *Aplysia* neurons use peptides to communicate with their central and peripheral targets. We have raised antibodies to ELH, peptides A + B, ELH, $SCP_B$, R14, and L11 peptides, and have obtained an antisera directed against FMRFamide. Using these reagents, the distribution of immunoreactive cell bodies and processes have been determined. These studies demonstrate that the individual peptides are localized in as few as 12 or as many as 200 central neurons.

We are using EM immunohistochemical techniques to study the subcellular distribution of the peptide products. Ganglia are fixed in increasing paraformaldehyde to 8%, single neurons dissected and prepared either for ultrathin frozen sectioning by embedding in gelatin and 2.3 M sucrose, or dehydrated and embedded in Lowicryl K4M. Thin sections collected on grids with either method are reacted with primary antisera, and visualized using protein A coated gold colloid. The L11 and R14 peptides and $SCP_B$ are localized in dense core vesicles. The R14 peptide is also contained in granular (not as dense) vesicles as revealed by the Lowicryl procedure. Studies to determine the localization of the other peptides are in progress.

Double labeling experiments using antibodies directed against different regions of the R14 peptide precursor have also been performed. In this procedure, tissue sections are reacted with a primary antisera followed by protein A coated 12 nm gold colloid; after blocking all unreacted antisera, a primary antibody directed against a different region of the precursor is reacted with the tissue, followed by protein A coated 6 nm gold particles. Presence of both size particles in the same granules demonstrates that different regions of the R14 peptide precursor are in the same vesicles.

These data imply that many *Aplysia* neurons synthesize large amounts of a specific peptide precursor which is subsequently packaged into dense core and granular vesicles.

234.2 IMMUNOHISTOCHEMICAL LOCALIZATION OF HISTAMINE IN THE BRAIN AND PERIPHERAL NEURONS AND ENDOCRINE CELLS. P. Panula, L. Kivipelto*, M. Kaartinen*, O. Häppölä* and J.-Y. Wu¤. Department of Anatomy, University of Helsinki, Siltavuorenpenger 20, 00170 Helsinki, Finland and ¤Department of Physiology, Penn State University, Hershey, PA 17033, USA.

Antisera against histamine coupled to hemocyanin with carbodiimide were used to locate neurnoal histamine in the brain and peripheral nerves immunohistochemically. In the rat brain, histamine-immunoreactive neurons were found only in colchicine-treated rats. They were located in the tuberal, caudal and postmammillary caudal magnocellular nuclei. Some of these neurons exhibited L-glutamate decarboxylase-immunoreactivity as well. This indicates that some GABAergic neurons may contain histamine. Histamine-immunoreactive nerve terminals were detected in many brain areas. They were numerous in the paraventricular and supraoptic nuclei of the hypothalamus. Moderate densities of nerve terminals were seen in the cerebral cortex, neostriatum and hippocampus.

Only histamine-immunoreactive mast cells were found in the posterior pituitary gland. Lack of immunoreactive nerve fibers may indicate that histamine regulates the pituitary gland at the level of hypothalamic nuclei, where numerous nerve fibers are found.

Small intensily fluorescent cells (SIF cells) in the guinea pig and rat sympathetic ganglia were immunoreactive for histamine. Some histamine-immunoreactive chromaffin cells were detected in the adrenal medulla of the rat, whereas numerous process-bearing cells in the guinea pig adrenal medulla were found.

Nerve fibers in the rat ileum exhibited histamine-immunoreactivity. These fibers were located mainly in the submucosal ganglion cell layer, where immunoreactive nerve cell bodies were also found after local colchicine treatment. Fewer fibers were seen in the myenteric plexus.

Numerous enterochromaffin-like cells in the rat stomach were immunoreactive for histamine. Positive mast cells were also found. Fewer cells were seen in the guinea pig stomach. In both species, the immunoreactive enterochromaffin-like cells were found in the oxyntic gland area.

The histamine-immunoreaction was blocked completely by preabsorption with histamine but not with L-histidine, histidine-containing peptides, catecholamies or serotonin. Affinity-purified antibodies stained the same structures as the crude antiserum.

The results show that histamine is widely distributed in the central and peripheral nervous system and endocrine cells, where in may function as a neurotransmitter or hormone.

Supported by grants from the Sigrid Jusélius Foundation and the Paulo Foundation.

234.3 IMMUNOCHEMICAL AND IMMUNOCYTOCHEMICAL CHARACTERIZATION OF RAT HISTIDINE DECARBOXYLASE. S. Levine, D.H. Park, G. Teitelman and T.H. Joh. Dept. of Neurology, Cornell Univ. Med. Coll., New York, NY 10021.

Histidine decarboxylase (HDC) catalyzes the conversion of L-histidine to histamine in a single biosynthetic step. Several lines of evidence suggest that histamine is a neurotransmitter in the central nervous system. In the present study, we used immunochemical and immunocytochemical techniques to characterize HDC.

HDC was purified from fetal rat liver (17-18 days of gestation). The purification procedure included homogenization in 3 volumes of 100 mM potassium phosphate buffer containing 1 mM DTT, 1 mM EDTA and 10 uM pyridoxal phosphate, centrifugation at 100,000g for 1 h, 25-45% ammonium sulfate fractionation of the supernatant followed by successive column chromatographies on DEAE-cellulose, chromatofocusing on polybuffer exchanger 94 (Pharmacia) and Bio-Gel A-0.5m. These purification steps provided a more than 2,000-fold enrichment in the specific activity of the enzyme and a 20% recovery of the total enzyme activity.

The final purification step involved preparative polyacrylamide gel electrophoresis. The enzymatically active protein extracted from the gels showed a protein band with a subunit molecular weight of 54,000 daltons on SDS-PAGE.

Antibodies were raised against HDC in rabbits by injecting the enzymatically active enzyme eluted from polyacrylamide gel slices. The antibodies were determined to be specific for HDC by several immunochemical criteria. The antisera precipitated HDC activity from a crude enzyme preparation. In Western blot analysis, the antibody immunoreacted with a single 54,000 dalton protein in both crude and partially purified preparations of fetal rat liver.

Using peroxidase-antiperoxidase immunocytochemistry, numerous cells were labelled in the caudal magnocellular and dorsomedial hypothalamic nuclei which extended into lateral, perifornical, posterior and supramammillary substructures. This finding is consistent with the biochemical and immunofluorescent localization of HDC as reported by others. In addition, HDC-like immunoreactive cells were localized in fetal rat liver and stomach of adult rats.

This antibody probe will be used to study the regulation of HDC at the molecular level as well as the anatomy of the histamine system. (Supported by Grant numbers MH24289 and NS 19002. S. Levine is the recipient of a scholarship from the Inst. Invest. Clin., Maracaibo, Venezuela.)

234.4 COLOCALIZATION OF TYROSINE HYDROXYLASE AND PROTEIN-O-CARBOXYLMETHYLTRANSFERASE IN MONOAMINERGIC NEURONS OF THE RAT BRAIN. D.M. Kuhn*, M.L. Billingsley, and C.D. Balaban*, Sec. Biochem. Pharmacol. NHLBI, NIH, Bethesda, MD 20205, and Hershey Medical Center, Pennsylvania State University, Hershey, PA 17033.

In an attempt to determine whether protein carboxylmethylation plays a role in monoaminergic neurons, we have used immunocytochemistry to demonstrate the presence of both protein-O-carboxylmethyltransferase (PCM) and tyrosine hydroxylase (TH) in the same neurons of rat brain. Tyrosine hydroxylase was purified from rat pheochromocytoma cells (PC-12) using ammonium sulfate fractionation, DEAE anion exchange-chromatography, Sepharose-4B molecular sizing and heparin-Sepharose chromatography. TH was homogenous after SDS gel electrophoresis; this fraction was used to produce polyclonal antibodies in rabbits. PCM was purified to homogeneity using DEAE anion exchange and S-adenosylhomocysteine-agarose chromatography and molecular sizing. Polyclonal antibodies were generated in rabbits using gel slices of purified PCM as antigens. Western immunoblot analysis indicated that the antisera against tyrosine hydroxylase recognized only one band of 58,800 daltons which corresponded to the purified subunit of TH. The TH antisera recognized this 58,800 dalton subunit in PC-12 cell extracts, adrenal chromaffin cell extracts, and rat brain striatal homogenates; it did not crossreact with purified phenylalanine hydroxylase nor PCM. PCM antisera recognized a 27,000 dalton subunit corresponding to purified PCM which was also present in brain cytosol, striatal cytosol and additional brain regions; it did not crossreact with either purified or partially purified preparations of TH. Neither antisera appeared to react with any other band in any preparation examined. Both antisera were used to immunolocalize TH and PCM in formalin-fixed sections of rat brain. TH antisera recognized neurons in the substantia nigra, locus coeruleus, brainstem nuclei, and several other discrete regions corresponding to catecholamine neurons. The TH antisera was able to discern fine axonal processes and could be used to trace the nigrostriatal tract. Numerous projection fibers which were TH positive could be seen in the cortex and the cerebellum. Antisera against PCM recognized neurons throughout the rat brain; however, high levels were detected in the substantia nigra and the locus coeruleus. When serial sections were immunostained for TH and PCM, the highly immunoreactive PCM-containing cells were found to be positive for TH. The high levels of both PCM and TH in monoaminergic cells of the rat brain are consistent with studies suggesting that PCM can modulate neurotransmission in monoaminergic neurons. Studies are underway to determine if PCM activity can directly alter TH activity both in vitro and in vivo.

234.5 SUBSTANCE P-LIKE IMMUNOREACTIVITY IN THE SPINAL TRIGEMINAL NUCLEUS OF THE CAT. J.P. Drew*, L.E. Westrum, and R.H. Ho. (SPON: A. B. Harris) Depts. of Biological Structure and Neurological Surgery, Univ. of Wash., Seattle, WA 98195 and Dept. of Anatomy, Ohio State University, Columbus, OH 43210.

Light microscopy (LM) is being used to study the patterns of substance P-like immunoreactivity (SPLI) in the brain stem spinal trigeminal nucleus of the adult cat. The peptide substance P (SP) an identified gut and sialagogic transmitter has also been implicated as an excitatory neurotransmitter. Commercially obtained antibody to SP (Immunonuclear) is being used along with anti-SP prepared according to the protocol of R.H. Ho. The indirect peroxidase anti-peroxidase (PAP) method for immunocytochemistry is being utilized following the protocol of Sternberger. In subnucleus caudalis (SC), heavy immunoreactivity is observed in a continuous lunate distribution extending into lamina I and II, agreeing well with previous studies of this region and the analogous substantia gelatinosa of the spinal cord dorsal horn. In caudal subnucleus interpolaris (SI) the immunoreactivity is observed in dorsal and ventral patches or accumulations dramatically different from the pattern observed in SC. The patterns of immunoreactivity in both SC and SI are surprisingly roughly coincident with those areas identified as receiving dental afferents. The area of transition between the two distinct patterns of immunoreactivity observed in SC and SI appears to be just caudal to the obex. In rostral SI the dorsal and ventral patches of immunoreactivity are observed to gradually diminish and then disappear with the ventral accumulation being slightly more persistant. Irregular accumulations are seen in subnucleus oralis (SO). Besides the more dominant immunoreactive terminals described above, transversely oriented immunoreactive fibers are observed. These fibers tend to predominate dorsally and ventrally in SC, are especially delineated at the SC-SI transition (obex), are scattered in SI and are seen in very small pockets in SO. Our results provide the first detailed description of the pattern of SPLI throughout the entire brain stem spinal trigeminal complex. Since the observed patterns of immunoreactivity have certain similarities to those areas receiving dental afferents, this study will form a necessary control for the analysis of possible changes in this distribution following dental lesions.

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234.6 IMMUNOCYTOCHEMICAL STAINING OF VASOPRESSIN BINDING SITES IN THE RAT BRAIN. R. Ravid*, D.F. Swaab*, T. v.d. Woude* and G. Boer* (SPON: European Neuroscience Association), Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam ZO, The Netherlands.

Vasopressin (VP) is produced by neurons, released into the bloodstream in order to regulate kidney function as a neurohormone and transported by axons to a great number of brain areas, where it acts as a neurotransmitter or neuromodulator.

An immunocytochemical procedure was developed to localize VP binding sites in the Brattleboro rat (di/di) brain, which is genetically deficient for VP but responds to the neuropeptide. The procedure comprises continuous in vivo administration of VP for a period of two weeks into the lateral ventricle, by means of small Accurel (microporous polypropylene) implants, loaded with 0.15, 1.5 and 15 µg of VP.

VP binding sites were localized immunocytochemically, with a higher resolution than the hitherto described autoradiographic techniques, in two distinct forms: (a) in perineural structures, e.g., in the dorso-rostral part of the lateral septum, striatum, cingulate cortex, the dentate gyrus of the hippocampus and cerebellar Purkinje cells, all mainly in the high dose loaded implants; (b) in neural cell bodies, e.g., in the lateral and medial septum, striatum, cingulate cortex, bed nucleus of the stria terminalis (BST), organum vasculosum of the laminae terminalis (OVLT) and locus coeruleus, all mainly in the low dose loaded implants.

The distribution of VP binding sites only partly overlaps with the sites of VP innervation. The difference in type of staining related to dose might mean that the current immunocytochemical procedure retains both low and high affinity populations of binding sites which may represent two different central effects of VP.

234.7 NADPH DIAPHORASE ACITIVITY IN THE POSTERIOR PITUITARY: RELATION TO FUNCTIONAL ACTIVITY. S. M. Sagar and D.M. Ferriero*, Neurology Service, Veterans Adminstration Medical Center and University of California, San Francisco, CA 94121.

NADPH diaphorase histochemistry stains selective populations of neurons in various regions of the brain (Vincent et al., J. Comp. Neurol. 217: 252-263, 1983). Among the cell populations stained by this method are the magnocellular neurons of the rat hypothalmus, along with their axons in the median eminence and terminals in the posterior pituitary. Since other oxidative enzymes, including cytochrome oxidase and succinic dehydrogenase, have been shown to vary in activity in response to alterations of functional activity, we investigated the possibility that NADPH diaphorase activity also reflects neuronal activity.

Adult, male Long Evans rats were given 2% saline to drink. This regimen has been shown to result in secretion of up to 90% of the stores of oxytocin and vasopressin from the posterior pituitary and to increase the rate of synthesis of the posterior pituitary hormones 4 fold (Gainer et al., J. Cell Biol. 73: 366-381, 1977). Control animals had free access to water. After 7 days, the animals were sacrificed and the posterior pituitary glands were assayed for NADPH diaphorase and protein.

The NADPH diaphorase assay, like the histochemical procedure (Scherer-Singler et al., J. Neurosci, Meth. 9: 229-234, 1983), measures the NADPH-dependent reduction of nitro blue tetrazolium dye (NBT). Aliquots of posterior pituitary homogenates are added to reaction mixture containing 0.05 M Tris-HCl, pH 8.0, 1 mM EDTA, 0.2% Triton X-100, and 0.2 mM NBT. After allowing endogenous substrates to be consumed, the reaction is started by adding NADPH to a final concentration of 0.5 mM. Reduction of NBT is monitored spectrophotometrically at 535 nm.

The mean specific activity of NADPH diaphorase in control rats was found to be 0.54 $\pm$ 0.02 µmol/min/mg protein compared to 0.80 $\pm$ 0.04 µmol/min/mg protein in salt-loaded rats ($p < 0.01$, Mann-Whitney U test, n=10.) There was also a 8% decrease in the mean protein concentration of the posterior pituitaries of the salt-loaded animals, presumably reflecting the diminished hormone stores, but this fails to account for the 47% increase in enzyme specific activity.

Therefore, the activity of NADPH diaphorase, like other histochemical markers of oxidative metabolism, responds to changes in functional activity of neurons. Since NADPH diaphorase, unlike other oxidative enzymes, is also a marker for neuronal populations containing specific neurotransmitter candidates, NADPH diaphorase offers the opportunity to assess the functional activity of certain chemically defined neuronal populations.

234.8 STELLATE NEURONS IN THE TURTLE DORSAL CORTEX CONTAIN $\gamma$-AMINOBUTYRIC ACID AND ITS SYNTHETIC ENZYME. M. Blanton*, J. Shen and A.R. Kriegstein. Dept. of Neurology, Stanford University Med. School, Stanford, CA 94305.

Recent intracellular studies of the dorsal cortex of the turtle, Pseudemys scripta, have demonstrated that the morphologically distinct pyramidal and stellate neurons have characteristic and distinctive membrane properties and synaptic responses (Kriegstein and Connors, Soc. Neurosci. Abst. 10:659). Afferent stimulation excites stellate cells and produces powerful $\gamma$-aminobutyric acid (GABA)-mediated inhibition of pyramidal cells. In order to support the role of stellate neurons as the mediators of this intrinsic cortical inhibition, we used Golgi and immunohistochemical techniques to study dendritic and axonal morphology and to verify that the stellate neurons utilize GABA.

Golgi-impregnated pyramidal and stellate neurons are morphologically distinct. Pyramidal cells have polymorphic or pyramidal somata located in or immediately subjacent to the pyramidal cell layer. Their spine-laden apical dendrites ascend obliquely through the molecular layer to the pial surface while spiny basal dendrites descend through the subcellular zone; axons descend from the soma or a proximal basal dendrite and issue recurrent collaterals. Stellate neurons have multipolar or fusiform somata and aspiny or sparsely spiny dendrites that are often beaded. The dendrosomatic axes of fusiform cells are predominantly horizontal with some oblique or vertical forms present. Horizontal neurons often possess one or more ascending secondary dendrite(s) positioned to allow reception of thalamic afferent excitation in the superficial molecular layer. Axons arise from a proximal dendrite or a soma-dendritic junction and arborize extensively within the layer containing the stellate cell somata. Axonal arborizations of stellate neurons within the pyramidal layer envelope neighboring pyramidal cell somata.

Antibodies directed against the inhibitory transmitter GABA and its synthetic enzyme glutamic acid decarboxylase (GAD) and a histochemical technique which reveals the GABA degradative enzyme GABA-transaminase all label the somata and proximal dendrites of stellate interneurons in all cortical layers. Pyramidal somata, while completely devoid of label, are outlined by darkly staining punctate structures, presumably the terminals of GABAergic interneurons within this layer. Similar presumptive terminals are found in all layers. Therefore, aspiny or sparsely spiny interneurons with locally ramifying axons contain GABA and form a distinct class of neurons which are positioned to mediate the intrinsic inhibition observed in physiological studies of the turtle dorsal cortex.

We thank Donald Schmechel for providing the GAD antiserum. This work was supported by NIH Grants NS00887-01 and NS21223-01.

234.9 SUBSTANCE P (SP) AND VASOACTIVE INTESTINAL PEPTIDE (VIP)-CONTAINING NERVE FIBERS REACH THE OVARY BY TOTALLY INDEPENDENT ROUTES. W.L. Dees*, C.E. Ahmed* and S.R. Ojeda* (SPON:R. Stillman). Dept. of Physiology, Univ. of Texas Health Science Center at Dallas, Dallas, TX 75235.

We have previously shown that the immature ovary is innervated by both SP-and VIP-ergic fibers (Dees et al., Biol. Reprod., in press; Ahmed et al., Endo. Soc., Abs., 1985). To determine the origin of these fibers, juvenile 24-day-old rats were either sham-operated or subjected to transection of either the abdominal vagus nerves (AV), superior ovarian nerves (SON), plexus nerves (PN), or to concomitant AV-SON transection. Six days later their ovaries were fixed in Zambonies fixative and processed for immunohistofluorescent examination of their SP and VIP-ergic innervation. Staining was performed on frozen 10 µm sections using specific antisera (anti-SP Kozlowski #317, and anti-VIP Abe-Reichlin #750) followed by fluorescein isothiocyanate-labelled goat anti-rabbit IgG. Sectioning of the AV affected neither the SP-nor the VIP-ergic innervation of the ovary. In contrast, sectioning of the SON or both the SON and AV nerves completely eliminated VIP-positive fibers from all ovarian compartments innervated by the peptide (i.e. interstitial tissue, follicles and blood vessels) without affecting SP innervation. Sectioning of the PN, however, selectively eliminated SP positive fibers. These results demonstrate that VIP innervation of the ovary is via the superior ovarian nerve, whereas SP innervation is via the plexus nerve. This remarkable anatomical localization is consistent with our view that both peptides regulate ovarian activity by acting on different functional components of the gland.

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234.10 ENKEPHALIN-IMMUNOREACTIVITY IN THE RAT SUPERIOR CERVICAL GANGLION. S. Soinila, H. Päivärinta, O. Häppölä* and P. Panula. Department of Anatomy, University of Helsinki, Siltavuorenpenger 20 A, SF-00170 Helsinki, Finland

Enkephalin-immunoreactive nerve cells and fibres are known to be present in the superior cervical ganglion (SCG) of the rat (Schultzberg et al., Neurosci 1979:4: 249). The purpose of the present study was to examine the origin and projections of these fibres, as well as presence of enkephalin in cultured sympathetic ganglia.

The indirect immunofluorescence method of Coons was applied on cryostat sections or on explants of SCG after fixation with 4 % paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.4. Specific antisera to both met-enkephalin$^{5}$-arg$^{6}$-phe$^{7}$ (MEAP) and met-enkephalin$^{5}$-arg$^{6}$-gly$^{7}$-leu$^{8}$ (MEAGL) were used.

Some principal nerve (PN) cells were immunoreactive to MEAP and MEAGL. MEAP-immunoreactive fibres were seen to extend from some PN cells. Small intensely fluorescent (SIF) cells were non-reactive. Immunofluorescent fibres enclosed both PN cells and SIF cells. The number of these fibres decreased after section of the preganglionic trunk. Ligation of the preganglionic trunk resulted in accumulation of immunofluorescent material on both sides of the ligature. Some immunoreactive cells and nerve fibres were observed in the stellate ganglion. No immunoreactive cells were found in the spinal cord at the levels C8-Th3. Ligation of the postganglionic nerves resulted in an increased number of immunoreactive nerve fibres on the ganglionic side of the ligature, irrespective of section of the preganglionic trunk. Some immunoreactive nerve fibres were present in the SCG even after combined pre- and postganglionic nerve division. Cultures of the SCG showed that the PN cells maintain MEAP immunoreactivity in vitro. MEAP- and MEAGL-immunoreactive varicose fibres were observed in the fibre outgrowth.

The present observations indicate that (1) the rat SCG contains both extrinsic and intrinsic enkephalin-immunoreactive fibres; (2) both PN cells and SIF cells may receive innervation by enkephalin-containing nerve fibres; (3) ganglionic neurons projecting to the sympathetic target tissues contain enkephalin.

234.11 IDENTIFICATION OF NEUROHEMAL-ENDOCRINE AND SYNAPTIC-NEUROACTIVE GONADOTROPIN RELEASING HORMONE (GnRH) IMMUNOREACTIVE NEURONS IN MOUSE BRAIN. L. Jennes and W.E. Stumpf. Dept. of Anatomy, University of North Carolina, Chapel Hill, NC 27514.

A combination of immunofluorescence for GnRH with simultaneous visualization of retrogradely transported horseradish peroxidase, injected intravenously, was used to identify populations of GnRH neurons in the mouse which have access to fenestrated capillaries. An average of about 530 immunoreactive GnRH neurons was found, most of which are present in the septum, including the neurons of origin of the nervus terminalis, the nucleus medialis septi, the nucleus triangularis septi, and the nucleus tractus diagonalis throughout its caudal extent. GnRH neuronal perikarya can also be seen in the lateral anterior hypothalamus, the nucleus preopticus medianus and medialis and the rostral nucleus periventricularis hypothalami. In addition, single GnRH neurons occur in the nucleus supraopticus, the bed nucleus of the stria terminalis and the cingulate cortex. The mediobasal hypothalamus does not contain GnRH neurons.

GnRH neurons which take up intravenously injected horseradish peroxidase from the perivascular space of fenestrated capillaries are located in all of the above areas, intermingled with GnRH neurons which do not take up horseradish peroxidase. A preferential topographic accumulation of neurohemal GnRH neurons could not be detected. Under maximal stimulation of GnRH secretion after two weeks of castration, 65% of all GnRH immunoreactive cells had taken up blood borne horseradish peroxidase, while after a 3-day estradiol pretreatment only 35% of all GnRH positive neurons had taken up the enzyme.

While the existence of GnRH neurons with a dual action via collaterals cannot be excluded, the results suggest that two GnRH populations exist, one with access to fenestrated capillaries which is related to neurosecretory endocrine regulation of anterior pituitary gonadotropin secretion, and one without access to fenestrated capillaries which is related to intracerebral neurotransmission only.

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234.12 SOMATOSTATIN-LIKE IMMUNOREACTIVITY IN THE MIDBRAIN OF THE CAT. K.M. Spangler*, B.J. Morley, (SPON: E. Javel) Boys Town National Institute, Omaha NE, 68131

Somatostatin (SS), like many other neuropeptides originally described in the hypothalmus or periphery, has been shown to have a wide distribution in the central nervous system. In a previous study (Morley et al., Dev. Brain Res., in press), we examined SS-like immunoreactivity in the interpeduncular nucleus of young and adult cats. During the course of that study, we noticed some major differences in the distribution of SS-like immunoreactivity in the midbrain, as compared to the rat (Finley et al., Neurosci. 6, 1981, 2173-2192). The material was prepared with a PAP immunohistochemical technique. The primary antibody had been raised against synthetic cyclic SS conjugated to keyhole limpet hemocyanin (Immunonuclear Corp.). In addition to the interpeduncular nucleus, other midbrain nuclei containing cells with SS-like immunoreactivity included the superior colliculus, the lateral reticular formation, the parabigeminal nucleus, the lateral wings of the dorsal nucleus of the raphe, the dorsal subnucleus of the periaqueductal gray, the pretectal complex, the nucleus cuneiformis, the rostral pole of the inferior colliculus, the nucleus of the brachium of the inferior colliculus, and the reticular nucleus of the thalamus. In the superior colliculus, a large number of small neurons in the superficial gray layer contained SS-like immunoreactivity. Additionally, clusters of small cells in the deeper portions of the intermediate gray layer were so labeled. Fibers with dense SS-like immunoreactivity were found in the periaqueductal gray, along the raphe, and in the ventral and lateral tegmentum. Cells surrounded by SS-immunoreactive terminals were present within portions of these areas. These findings suggest a role for somatostatin in the tecto-parabigeminal system and in other midbrain pathways.

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234.13 ORIGIN OF MOTILIN IMMUNOREACTIVE FIBERS IN RAT MEDIAN EMINENCE. G. Nilaver, R. Bodnar, and E. A. Zimmerman. Dept. Neurology, Oregon Health Sci. Univ., Portland, OR 97201, and Dept. Psychology, Queens College, CUNY, Flushing, NY 11367.

Growth hormone releasing factor (GRF) has been implicated in the hypothalamic regulation of pituitary growth hormone (GH) secretion. Lesion studies with monosodium glutamate (MSG) furthermore, have revealed neurons in the arcuate nucleus (ARC) to be the primary source of the GRF somatotrophic fibers that project to the median eminence (ME) (Bloch et al., Nature, 307: 272, 1984). Motilin has also been demonstrated in the hypothalamus by immunohistochemistry and RIA, and shown to facilitate the in vivo and in vitro release of GH in rats (Samson et al., Brain Res Bull, 12: 57, 1984). Motilinergic perikarya in rat hypothalamus have been localized to the preoptic, ventromedial (VMH), and ARC regions, and immunoreactive fibers have been described in the zona externa of the ME (Jacobowitz et al., Peptides, 2: 479, 1981). The precise contribution of the ARC to the motilin immunoreactivity in rat ME however, has not been fully established. Since neonatal administration of MSG produces neurotoxic degeneration of the MBH and ARC (Olney, Science, 164: 719, 1969), and depletes ARC GRF immunoreactivity (Bloch et al., Nature, 307: 272, 1984), we studied the distribution of motilin in the hypothalamus of MSG treated rats and saline controls, employing immunocytochemistry. A few animals from each group were colchicine treated prior to sacrifice. Brains were immersion fixed in Bouins fixative. Hypothalamic sections (100 um; coronal and sagittal) were immunoreacted with a specific rabbit antiserum to synthetic porcine motilin, employing biotinylated protein A with avidin-biotin peroxidase in the preembedding staining technique. Adjacent sections were immunoreacted with an antiserum to rat GRF (generous gift from Dr. C. Hollander). In colchicine treated controls, motilin immunoreactive perikarya were found in the preoptic, VMH and ARC regions. MSG treated rats showed total elimination of GRF immunoreactivity in the ME and ARC, confirming the extent of the MSG lesion. The motilin staining in the ME and preoptic regions of these rats however, was not affected, and was identical to that seen in saline controls. This finding, and the absence of motilin imunoreactivity in the ARC and VMH of MSG rats even upon colchicine pretreatment, suggests perikarya in the preoptic area to be the major source of the motilin immunoreactivity in the ME. Since we find the MSG effect to be selective for GRF furthermore, this model provides an elegant system for studying the relative importance of hypothalamic motilin in the pituitary release of GH. (Supported by NIH grant NS18324).

235.1 DYNAMIC BEHAVIOR IN INITIAL ORDERING OF THE RETINOTECTAL MAP AS REVEALED BY A VITAL-DYE FIBER-TRACING TECHNIQUE.
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In the visual system of Xenopus laevis, the axons from the retinal ganglion cells of the eye form a topographic projection onto the optic tectum. The order of the projection is highly regular both during development and after regeneration. Many recent studies have focused on revealing possible mechanisms for this precise order. In contrast to the static view of the system that one might expect from examining the regularity of the projection, some studies indicate that part of the patterning process involves the dynamic behavior of optic fibers. For example, during regeneration, a roughly ordered projection forms initially, which is then refined with time. We have used a newly developed fiber tracing technique to follow the early ingrowth of the optic nerve fibers into the tectum and have found further evidence for dynamic behavior in the initial development of topography in the retinotectal projection.

Previously, anatomical and electrophysiological techniques have been used to obtain static views of development which then must be compiled to provide a glimpse of dynamic behavior. In our study, we have refined a fluorescent dye fiber-tracing technique which allows us to view neuronal projections in living Xenopus embryos. This offers the possibility of following the development of the retinotectal projection in individual animals over a period of days, and thus permits direct observation of the dynamics of neuronal patterning. The technique utilizes fluorescent dextrans, vital dyes, which are microinjected into Xenopus embryos at the one cell stage. At tailbud stages (st 32) labeled eyebud tissue is grafted into unlabeled hosts. The fluorescent dextran fills the axons of the developing retinal ganglion cells, and as the head of the developing larva becomes transparent, growth of the labeled neurons into the tectum can be followed in situ with an epifluorescence microscope.

In order to observe the initial appearance of topography in the projection pattern we grafted labeled half eyebuds into unlabeled hosts. An image intensifying camera was used to repeatedly record the projection pattern in individual animals for the next 2 weeks. Experiments using this technique reveal that dorsal and ventral optic nerve fibers sort out into an ordered projection early in development; in contrast, nasal and temporal fibers initially overlap, then sort out into the adult pattern over a period of days. Thus, patterning of the nasotemporal dimension of the retinotectal projection involves a dynamic process in which fibers gradually sort out to innervate different areas of the developing tectal neuropil.

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235.2 **AFFERENT INDUCED ALTERATIONS IN THE DENDRITIC DOMAINS OF TECTAL NEURONS IN THREE-EYED FROGS.**
**L.C. Katz and M. Constantine-Paton Neurobiology Lab. The Rockefeller University, N.Y.N.Y. 10021 and Biology Dept., Yale University, New Haven CT 06511.**

**Recent evidence supports the idea hat topographical map formation involves a phase during which afferent synapses sort according to the proximity relationships among the presynaptic cell bodies. Proposed mechanisms for the phenomenon postulate that the effect is mediated via post-synaptic cells which stabilize groups of synapses on the basis of temporal correlations of their activity. Considerable information on activity-dependent modifications of afferents is available, however little is known about the effects of such modifications on post-synaptic cells. The eye-specific tectal stripes of three-eyed frogs are distinct morphological indicators of zones of correlated synaptic input. We have developed an in vitro tectal preparation which allows us to visualize both afferent stripes and post-synaptic cells. Tetramethylrhodamine isothiocyanate (TRITC), an anterograde tracer, is injected into the vitreous of one eye. Three to five days later the tecta are surgically removed and maintained in cold HEPES buffered glucose supplemented Ringer's solution, in a saturated oxygen atmosphere. Individual tecta are transferred to a recording chamber on the stage of a fluorescence equipped compound microscope for intracellular recording. Under 100X magnification, TRITC stripes are clearly visible, separated by distinct interstripe zones. Lucifer yellow (LY) filled microelectrodes (20% LY in 0.1 M LiCl, 250Ω resistance) are used to impale and fill tectal neurons (1-2 nA, 1-3 min.).**

**Fixed tecta were prepared as wholemounts, and patterns of dendritic arborizations of filled neurons were related to the pattern of labeled stripes. On this basis we distinguished three broad groups of tectal neurons. The first consisted of cells whose highly branched, bushy dendrites (50-100μm diameter dendritic fields) respected the boundaries between stripes and interstripes, often abruptly terminating or bending around such borders. The other two cell groups had widespread, sparsely branched dendrites extending over distances greater than the width of individual stripes (approx. 150μm), and often over several stripes. In one group, the majority of dendritic branches were preferentially located within the stripes of one eye. Soma position was not consistently related to this branching pattern. The final group included neurons with very large (>400 μm) dendritic fields. Such cells show dendritic arborization in both stripe and interstripe zones. In many cases we traced axons for long distances, but in none of these groups did we observe extensive horizontal collaterals. These results suggest that at least some tectal neurons alter their dendrites in order to maximize the the amount of correlated input they receive.**
**Supported by NIH Grants EY05739 to LCK and EY01872 to MCP.**

235.3 EXPERIMENTALLY STABILIZED RETINAL PROJECTIONS TO THE HAMSTER VENTROBASAL COMPLEX ARE FUNCTIONAL.
D.O. Frost and C. Metin (SPON: R.J.J. Benson). Yale Med. Sch., New Haven, CT 06510

Retinofugal axons can form permanent, retinotopic connections in the somatosensory (ventrobasal, VB) nucleus of the Syrian hamster thalamus. This occurs when, in newborn hamsters, the superior colliculus and lateral geniculate body are ablated and alternative terminal space is created in VB by transection of the ascending lemniscal afferents to VB (Frost, D.O., J. Comp. Neurol., 203:227, 1981). The retino-VB projection occurs by the stabilization of an early, normally transient projection (Frost, D.O., Neurosci. Abs., 9:26, 1983) and forms synapses (Campbell, G. & Frost, D.O., this meeting). This study was undertaken to determine whether the anomalous, stabilized retino-VB projection is functional. In 15 hamsters operated as described above and 2 normal hamsters, all at least 10 weeks old, multi-unit recordings were made from the cortical targets of VB, the first and second somatosensory cortices (SI and SII, respectively). Since retinofugal axons terminate only in the diencephalon or mesencephalon, the strategy of recording in the cortex assures that the recording site is post-synaptic to retinofugal axons. Visual stimulation reliably evoked multi-unit responses in SI and SII of operated, but not normal hamsters. Most multi-unit receptive fields (RF's) in SI and SII had a single, clearly defined zone of maximal response, although some had two such zones. The mean area of the zones of maximal response in SI and SII was about 4.1 times the mean size of visual RF's recorded in area 17 of normal hamsters. The visual RF's in the somatosensory cortices were preferentially distributed in the lower visual field. In 7 cases we mapped the visual field representations in SI or SII. In SI and SII the lower temporal to upper nasal field axis was represented along the medial to lateral and posterior to anterior directions, respectively. The differences in orientation between the representations of this visual field axis in SI and SII are those that would be predicted given the differences in orientation between the somatic representations in normal rodent SI and SII. There was no obvious representation of the upper temporal to lower nasal field axis in SI or SII. The orderly cortical representation of only one visual field axis and the existence of RF's with two zones of maximal response are both probably due to the way in which the axes of the visual field representation in VB intersect lines of thalamocortical projection in VB. These results demonstrate that experimentally stabilized retino-VB axons can form functional synapses in VB. They also suggest that neural structures which normally process information specific to one sensory modality have the potential of mediating function for other modalities.

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235.4 NUMBER OF NEURONS IN THE MONKEY'S DORSAL LATERAL GENICULATE NUCLEUS DURING DEVELOPMENT
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The refinement of neural connections is often mediated by competitive interactions between axon terminals for targets or trophic molecules. In many cases the end result of this competition is the death of a large fraction of the initial complement of presynaptic neurons. For instance, the segregation of optic axons from right and left eyes in the dorsal lateral geniculate nucleus (DLGn) of fetal rhesus monkeys is associated with the loss of more than half of all retinal ganglion cell axons (Rakic & Riley, '83; Science **219**: 1441), and this loss is evidently due to ganglion cell death rather than the retraction of excess axon collaterals (Lia et al., in press). In the present study we have tested whether a similar process—the segregation of geniculate terminals into ocular dominance columns in striate cortex—is also associated with neuron death, in this case, in the DLGn.

Serial celloidin, polyester wax, and frozen sections of the DLGn of fetal, neonatal, juvenile, and adult monkeys (*Macaca mulatta*) stained with cresyl violet were examined using a 63X oil objective (n.a. 1.4) and Nomarski optics. At younger fetal ages—before embryonic day 90 (E90)—all cells in the DLGn were counted, but after E90, when glial cells became more numerous and could be readily distinguished from neurons, only the latter were counted. Fifty to 100 individual counts were taken at sites located at caudal, middle, and rostral levels extending from the dorsal to ventral surfaces of the nucleus through all laminae and interlaminar zones. A modified Abercrombie correction factor was used to determine average cell density. The volume of the DLGn—calculated from measurements of the cross-sectional area and the thickness of serial sections—was multiplied by the mean cell density to give the final estimate of cell number.

The estimates of cell number showed considerable variation between individuals. Those from fetuses between E70 and E96 ranged from 1,500,000 to 2,200,000 with an average of 1,950,000 (n=7). Estimates from postnatal animals ranged from 1,000,000 to 2,000,000. The average population of neurons in neonates and infants up to 2 months old was 1,410,000 (n=6) and did not differ significantly from the average in juveniles and adults up to 10 years old (1,490,000, n=5). The 20-30% loss of cells in the DLGn between midgestation and maturity may not be entirely due to neuron elimination since counts at the youngest ages (E70 to E87) probably included a small number of early-generated glial cells. These quantitative data are in harmony with observations on the number of necrotic cells in the DLGn: The incidence of pyknotic cells was merely 1 in 5000 from E45—when the last neurons destined for the DLGn are generated (Rakic, 1977, J. Comp. Neurol. **176**: 23)—until E60 (n=4). After E70 pyknotic cells were more common (up to 1 in 1000), and although they persisted during the neonatal period, they were not seen after the 2nd postnatal month. The small size of the pyknotic nuclei indicates that most belong to degenerating glial cells.

Taken together, the results demonstrate that number of neurons in the DLGn is relatively stable during the formation of cellular laminae, the segregation of retinogeniculate fibers, and synaptogenesis in the principal visual centers. Furthermore, since the number of neurons remains unchanged during the formation of ocular dominance columns early in infancy (Hubel et al., 1977, Phil. Trans. R. Soc. **278**: 377), the segregation of the initially diffuse geniculo-cortical projection is not associated with neuron death in the DLGn.

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235.5 THE EFFECT OF NEURAL ACTIVITY ON THE FORMATION OF GENICULATE CELL LAYERS. V.A. Casagrande, G.J. Condo and L.A. Durden*. Depts. of Anatomy and Psychology, Vanderbilt University, Nashville, TN 37232.

We investigated the role of retinal afferent activity on the development of cell layers in the lateral geniculate nucleus (LGN) of the tree shrew. In the tree shrew, all six layers develop postnatally. By postnatal day eight (P8), all interlaminar spaces (ILSs) are visible, although they continue to mature after this point. Our previous work has demonstrated that ILS formation is critically dependent upon the input from the retina. If the eyes are removed at birth, spaces between layers never form (Brunso-Bechtold and Casagrande, 1981).

In order to determine if ILS formation is dependent upon the activity of retinal axons, we injected eyes with tetrodotoxin (TTX) to abolish all activity or tried to selectively effect activity, either by injecting 2-amino-4-phosphonobutyric acid (APB) to block activity to the two On-center geniculate layers (1 and 2) or by manipulating illumination to the retina. The first group of tree shrews received either unilateral or bilateral intraocular (0.1 ug) TTX injections every other day from birth to P8-P14. This dose was twice that, by body weight, necessary to abolish activity for 2 days in adults. A second group of animals received either unilateral or bilateral intraocular (500 μM) injections of APB daily for the same period. A third group of tree shrews was reared from birth to P7 in: 1) continuous light, 2) continuous light with one eye patched, or 3) continuous darkness. At the end of each experimental period the animals were perfused, brains were cut, and LGN sections were stained with a Nissl stain or reacted for cytochrome oxidase. Results showed that manipulations of retinal illumination had little effect on LGN cell layer formation. Moreover, APB injections did not prevent the formation of the ILS between the On-center layers. However, APB injections did appear to slow the overall development of the LGN layers, i.e. the ILSs and the cells appeared immature compared with controls. TTX injections appeared to slow cell layer development more dramatically than APB, and in addition appeared to almost completely prevent the development of the ILS between the On-center layers: the remaining ILSs were all obvious though less mature than those of controls.

Taken together, these results suggest that retinal ganglion cell activity per se is not essential for the initial spacing of geniculate cells into layers but may be important for final maturation.

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235.6 POSTNATAL DEVELOPMENT OF SPATIAL CONTRAST SENSITIVITY OF NEURONS IN THE KITTEN DORSAL LATERAL GENICULATE NUCLEUS. J.S. Tootle and M.J. Friedlander. Dept. of Physiology and Biophysics, University of Alabama at Birmingham, Birmingham, AL 35294

The spatial processing capacities of neurons in the kitten dorsal lateral geniculate nucleus (dLGN) undergo a period of postnatal development. Two examples are spatial resolution (acuity) and spatial selectivity (reduced response to large stimuli) both which increase postnatally. A metric of choice for characterizing the spatial processing capacities of neurons is the spatial contrast sensitivity function (CSF), which yields several measures of spatial processing as well as a means for quantitative characterization of the receptive field profile. A goal of the present study is to provide the first description of the postnatal development of the spatial contrast sensitivity of dLGN neurons in the A-laminae of the normal kitten. We recorded the responses of dLGN neurons to drifting sinusoidal gratings varying randomly in contrast and spatial frequency. The intersection of contrast-response functions with spontaneous activity was used to estimate thresholds. We report preliminary results from 19 neurons (5 nonlinear, 14 linear) in 4-5 week old kittens and compare them to results from adult control animals (ours and from published reports - Mangel et al. J. Neurophysiol. 50:240-264, 1983, Lehmkuhle et al. J. Neurophysiol. 43:520-541, 1980). The maximum sensitivity, best spatial frequency and spatial resolution of averaged CSFs of kitten cells with linear and nonlinear spatial summation were lower than those of adult X- and Y- cells, respectively. Half the kitten cells with linear spatial summation had significant sensitivity roll-off at low spatial frequencies characteristic of adult X- cells; the remaining half had no such roll-off. Quantitative assessment of the low frequency roll-off was made using the contrast sensitivity ratio (CSR-sensitivity to .125 cycles/degree divided by maximum sensitivity). The mean CSR for kitten linear cells was .77, compared to .56 in the nondeprived lamina of 12 week-old monocularly deprived kittens and .20 in the adult. Kitten cells with nonlinear spatial summation, like Y- cells in the adult, had no low spatial frequency roll-off. These findings confirm our previous result, using the area-response technique, of a heterogeneity of maturity in the strength of surround antagonism in neonatal kitten dLGN. We are currently investigating the degree to which the immaturity of the antagonistic surround mechanism of indivudual dLGN cells reflects poorly developed inhibitory circuitry in the dLGN vs. a similar immaturity in the retina.

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235.7 MORPHOLOGY OF PHYSIOLOGICALLY IDENTIFIED RETINOGENICULATE AXONS IN CATS FOLLOWING BLOCKADE OF RETINAL IMPULSE ACTIVITY. M. Sur, P.E. Garraghty and M.P. Stryker. Section of Neuroanatomy, Yale Univ., New Haven, CT and Dept. of Physiology, UCSF, CA.

We are interested in the mechanisms by which retinal axons establish and maintain connections in the lateral geniculate nucleus (LGN). Blocking retinal ganglion cell activity with tetrodotoxin (TTX) in neonatal cats causes abnormal cell responses in the LGN (Archer et al., Science 217:743, 1982). In the present experiments, we investigated the effects of unilateral and bilateral blockade of retinal activity on the terminal morphologies of single retinal axons in the cat LGN.

For one set of experiments, cats were reared with chronic bilateral injections of TTX from 2 weeks of age, a procedure not expected to produce gross receptive field abnormalities in the LGN (Dubin and Stark, IOS 24:138, 1983). At 8-12 weeks, retinal axons were recorded, classified as X- or Y-cell, and injected intracellularly with HRP. X-cell axons in these bilaterally treated cats were found to be broader in terminal arbor than X-cell axons in normal adult cats or normal kittens at comparable ages (Sur et al., Nature 310:246, 1984). Importantly, X-cell axons were always found to be restricted to lamina A or A1 depending on their eye of origin. In contrast, Y-cell axons in bilaterally treated cats were narrower in terminal arbor than Y-cell axons in normal adult cats. Furthermore, some Y-cell axons crossed laminar borders and sprouted into adjacent laminae. Both X- and Y-cell axons appeared to have reduced bouton densities.

For the second set of experiments, cats were reared with unilateral injections of TTX from birth. In these animals, our sample of axons from the treated eye is still too low to permit generalizations. From the untreated eye, X-cell axons appeared normal in terminal morphology and bouton density, as did some Y-cell axons. Some Y-cell axons, however, sprouted into adjacent, treated, laminae.

During normal development, X-cell axons at 3-4 weeks of age have broad terminal fields in lamina A or A1 that get pruned into narrow arbors as Y-cell axons establish connections in these laminae and develop broad terminal arbors. Blockade of impulse activity may arrest the normal development of these arbors within laminae A and A1. The result that X-cell axons remain confined to the appropriate lamina while Y-cell axons sprout is consistent with similar results we obtain in cats enucleated at birth (Garraghty et al., Neurosci. Abst. 10:142, 1984). These studies suggest similar conclusions. X-cell axons establish connections in the LGN early and maintain laminar fidelity. Y-cell axons develop later and can sprout into adjacent denervated or electrically blocked laminae, unless prevented from doing so by normal afferent activity from the other eye.

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235.8 DEVELOPMENT OF RETINOGENICULATE AXON ARBORS FOLLOWING PRENATAL UNILATERAL ENUCLEATION. D.W. Sretavan, P.E. Garraghty, M. Sur and C.J. Shatz, Dept. of Neurobiology, Stanford Univ. Sch. of Med., Stanford, CA. and Section of Neuroanatomy, Yale Univ. Sch. of Med., New Haven, CT.

The terminal arborizations of retinogeniculate axons undergo stereotyped morphological changes during pre- and postnatal development, giving rise to the adult pattern by 3 months postnatally (Sretavan & Shatz, Nature 308:845, 1984; Sur et al., Nature 310:246, 1984). To further understand the factors controlling the development of ganglion cell arbors within the lateral geniculate nucleus (LGN), we have examined the effect of removing one eye at embryonic day 44 (E44: a time when inputs from the two eyes are extensively intermixed with each other within the LGN) on the subsequent morphology of axons.

In one set of experiments, retinal axons from the remaining eye were filled in vitro with horseradish peroxidase (HRP) at E59 (5 days before birth, a time when the eye-specific layers are normally present). Of the 15 axons reconstructed so far, 7 had expanded terminal arbors that included territory normally occupied by the enucleated eye. However, 6 others (both contra and ipsi to the remaining eye) appeared remarkably normal: The terminal arbors of all were restricted to the LGN region that normally would be appropriate to their eye of origin. Two other axons had terminal arbors intermediate in extent. To learn whether one class of axon is more susceptible to morphological modification by prenatal enucleation, axons were filled intracellularly with HRP at 6 postnatal months, when physiological identification is possible. Of the 5 axons recovered to date, 2 were Y-axons and 3 were X-axons based on a variety of tests. The X-axons were remarkably normal in morphology and had restricted terminal arbors similar in size and shape to normal, with two contralateral X-axons located approximately where layer A normally would have been, and the ipsilateral X-axon located where layer A1 would have been. In contrast, the two Y-axons were highly abnormal. Both were filled contralateral to the remaining eye, yet the terminal arbor of one was restricted to a region of the LGN that normally would have been ipsilaterally-innervated layer A1. The other Y-axon had an unusually extensive arbor located throughout what would have been layers A and A1.

These results demonstrate that the terminal arbors of retinogeniculate axons can expand as a consequence of prenatal enucleation. They further suggest that axons of different functional class may have different capacities, with Y-axons relatively susceptible and X-axons relatively immutable to modification.

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235.9 POSTNATAL DEVELOPMENT OF NEURONS WITH GABA IMMUNOREACTIVITY IN THE LATERAL GENICULATE NUCLEUS (LGN) AND VISUAL CORTEX OF THE CAT. R.E. Kalil, R.J. Wenthold and J. Zempel.* Dept. of Ophthalmology and Neurosciences Training Program, Univ. of WI., Madison; Lab. of Neuro-otolaryngology, NIH, Bethesda, MD. 20205; Dept. of Anatomy, Univ. of WI., Madison, WI. 53706.

Gamma amino butyric acid (GABA) is believed to be an inhibitory neurotransmitter in the retino-geniculo-cortical pathway in the cat In the LGN, GABA has been implicated as a transmitter that mediates receptive field surround antagonism, while in visual cortex GABA may play a role in determining the ocularity and selectivity of cortical neurons.

Physiological studies of the LGN and visual cortex in developing kittens have contributed an outline for the expression of these functional properties, but there is relatively little correlative evidence available concerning the development of the GABAergic neurons that are thought to be involved. We have therefore used direct immunocytochemical localization to identify GABA containing neurons in the LGN and visual cortex in cats of different postnatal ages.

The antibodies were made in rabbits against GABA conjugated to bovine serum albumin (BSA) as described by Storm-Mathisen et al. (Nature 301: 517, 1983). The antibodies were purified by affinity chromatography, and their specificity confirmed by immunoblotting.

Sections through the LGN and visual cortex were incubated with GABA antibodies at dilutions that varied from 1/500 to 1/4000, and immunoreactive neurons were then stained using the biotin-avidin method (Vector Laboratories). Cats with normal visual experience ranging in age from newborn to adult were studied. In addition, one cat raised with monocular deprivation for 9 months was examined.

In newborn cats, GABAergic neurons were not seen in the LGN, but were prominent in the adjacent perigeniculate nucleus. At 3 days postnatal, scattered, lightly stained neurons were evident in the LGN primarily in the C layers. By one week after birth, positive cell body staining was clear in the A layers of the LGN. Qualitatively, the density of GABAergic neurons appeared to increase gradually during the next two months, becoming mature between 8 and 12 weeks. Throughout development, cells in the LGN C layers were more densely labeled than those in the A laminae.

Cell somata were stained heavily in the visual cortex of the newborn kitten. Labeled neurons were spindle shaped with leading and trailing processes clearly visible. At 2 weeks postnatal, cell staining was restricted largely to cell somas, and at 3 weeks, stained cells, typically small in size, were distributed uniformly among all cortical layers. This laminar pattern was preserved in older kittens and adults, but in contrast with the LGN, GABAergic neurons in visual cortex developed a range of soma sizes and shapes.

Monocular deprivation from 7 days through nine months produced no obvious change in the pattern of labeled neurons in the LGN or visual cortex.

235.10 BIRTHDATES OF IMMUNOREACTIVE NEURONS IN THE FERRET VISUAL CORTEX. Jean D. Peduzzi, and T. L. Hickey. School of Optometry/The Medical Center, University of Alabama at Birmingham, Birmingham, Alabama 35294.

A combination of immunohistochemical and $^{3}$H-thymidine autoradiographic techniques were used to study the genesis of neuronal sub-populations in the visual cortex of the ferret. In a previous study (Soc. Neurosci. Abstr. 10: 519, 1984), we defined the overall time course of cortical neurogenesis in the ferret (E22-P14). In the present study, each ferret received a single injection of $^{3}$H-thymidine on one of the following days: embryonic day 26 (E26), E30, E34, E38, postnatal day 2 (P2), P6, or P10. Each animal was sacrificed at 2 months of age or older. Frozen sections (15 μm) were incubated in antisera (Immunonuclear) to gamma-aminobutyric acid (GABA) or cholecystokinin (CCK) and processed using the PAP technique. The resulting sections were then processed using standard autoradiographic procedures.

The production of GABA immunoreactive neurons extends throughout much of the period of cortical neurogenesis, a finding that correlates well with their ultimate distribution across all cortical layers. Further, a substantial proportion of those layer I cortical neurons that were generated early in the period of cortical neurogenesis (E26) were immunoreactive for GABA and/or CCK. These layer I neurons are unusual in that they do not follow the general inside-out pattern of cortical neurogenesis.

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235.11 MIGRATION AND FATE OF TRANSPLANTED VISUAL CORTICAL NEURONS. Susan K. McConnell. Vision Center, Salk Inst., La Jolla, CA 92037, and Neurobiology Dept., Harvard Med. School, Boston, MA 02115.

Neurons in a given layer of the mammalian cerebral cortex tend to have similar morphologies, projection patterns, and physiological properties; these cells are also generated at similar times during development. The relationship between cell birthdate and adult laminar position and connectivity raises the possibility that the ultimate fate of cortical neurons may be determined early in development, prior to migration. To address this question, a preparation has been established in which young cortical neurons from ferrets (which show a substantial period of postnatal neurogenesis) are dissociated and transplanted into host brains, where they migrate and differentiate side-by-side with host neurons.

Cells from the occipital cerebral ventricular zone of newborn ferrets were labeled with $^{3}$H-thymidine and a fluorescent dye (DAPI or Fast Blue), and were mechanically dissociated into a single-cell suspension. These cells were transplanted by pressure injection into the occipital ventricular zone of newborn host ferrets ("isochronic" transplants). Hosts were perfused after survivals of 2 hrs to 2 months, and their brains were processed for both fluorescence microscopy and autoradiography.

For short survivals (up to 12 days), transplanted cells were identified by their fluorescent marker. After 2 hrs survival, cells were clustered at the injection site in or near the ventricular zone. Here cells were round and resembled freshly dissociated cells in vitro. By 2 days, fluorescent cells had streamed radially into the intermediate zone; at these and later times, they were well integrated with cells of host origin. While in the intermediate zone, many cells resembled migrating cortical neurons: they had spindle-shaped nuclei and radially-extending leading processes. Some labeled cells had reached the cortical plate 4-6 days after injection. Here they underwent neuronal differentiation: the cells had oval or round nuclei (often with multiple nucleoli), and by 12 days had pyramidal cell bodies and long apical dendrites. A second class of fluorescent cell was also observed after short survivals. These had assumed positions in all regions of the cerebral mantle; they had round cell bodies and fine processes that radiated irregularly outward, and were presumably glial in nature. After long survivals (up to 2 months), transplanted cells were identified by $^{3}$H-thymidine autoradiography. Labeled cells had pyramidal neuronal morphology and were indistinguishable from surrounding host neurons. 90% of labeled cells were found in layer 2+3, the normal destination of neurons generated postnatally in the ferret.

These results show that transplanted cells respond normally to cues present in the host environment that guide or permit cortical neuronal development, and that transplantation per se does not alter the fate of cortical neurons. Using this preparation, neurons from fetal ferrets can be challenged to alter their fates upon transplantation into the brains of newborn hosts ("heterochronic" transplants). Thus the determinative influences of cell birthday and local environment on the ultimate laminar position and projections of a cortical neuron can be studied.

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235.12 CHARACTERISTICS OF TRANSIENT CORTICAL AFFERENTS TO VISUAL AREAS DURING POSTNATAL DEVELOPMENT IN THE KITTEN. J. Bullier*, C. Dehay* and H. Kennedy. Lab. Neuropsyschologie Experimentale, INSERM-U 94, 16 avenue du Doyen Lépine, 69500 Bron, France.

During its development the central nervous system is characterized by the formation of many connections which do not persist into adulthood. In the kitten one such category of temporary connections are those that link visual cortical areas with regions subserving other sensory modalities as well as motor cortex (Dehay et al. Exp. Brain Res.,57:208-212,1984; Innocenti and Clarke Dev. Brain Res.,14:143-148, 1984). This raises the question of whether these transient connections simply reflect a development stage of poor specificity and whether they may play a functional role. We have sought to answer this question by determining if there are precise boundaries to those regions which project transiently to areas 17, 18, and 19 and if the parent neurons have a specific laminar location.

Injections of the retrograde tracers fast blue or HRP-WGA were made in the grey matter of areas 17, 18, or 19 in kittens aged from 4 to 57 postnatal days. In each animal, the locations of labelled neurons were recorded by an X-Y plotter electronically linked to the microscope stage so as to delimit the entire extent of the cortical territories of origin of these temporary projections. In all animals below 35 days of age, we found retrogradely labelled neurons in limited regions of auditory and somatosensory cortex including primary areas AI and SI, in orbital cortex, limbic cortex and in smaller numbers in motor cortex. These cortical neurons have a particular laminar distribution. In the frontal cortex most of them are located in lamina I, whilst elsewhere at the boundary between laminae I and II and in upper III. This feature distinguishes them from other corticocortical neurons forming permanent callosal or associational connections which are located lower in layers II and III as demonstrated in double label experiments.

These results show that the transient cortical connections to the visual cortex in the kitten are specific in at least two aspects and suggest that they are not merely the result of errors during development. Firstly, they do not orignate from neurons evenly distributed throughout the fronto-parietal and temporal cortex but belong to precise cortical territories. Secondly these transient projections issue from a neuronal population which has a laminar location different from the population of neurons giving rise to stable connections, indicating perhaps differences in birth date and/or in the rate of migration. These specific characteristics would accord with the notion that transient projections may play a dynamic role during maturation of the cortex since it has been demonstrated (Dehay et al. 1984) that their terminals penetrate the grey matter and are in a position to interact with other cortical afferents.

235.13 DEVELOPMENT OF RESPONSE PROPERTIES OF POSTEROMEDIAL LATERAL SUPRASYLVIAN VISUAL CORTEX NEURONS IN THE CAT: IMPLICATIONS FOR FUNCTIONAL COMPENSATION. Maureen A. McCall*, Lillian Tong, and Peter D. Spear. Dept. of Psychology and Neurosciences Training Program, Univ. of Wisconsin, Madison, WI 53706.

Previous experiments have shown that physiological compensation occurs in posteromedial lateral suprasylvian (PMLS) cortex following early visual cortex (areas 17, 18, and 19) damage in cats (Tong, Kalil, & Spear, 1984). The critical period for the compensation ends between 12 and 26 weeks of age. In order to better understand the mechanisms of this compensation, the present study investigated the normal postnatal development of response properties of PMLS cortex neurons. Extracellular recordings were made in kittens at 2, 3, 4, 8, or 12 wks of age, with 3 to 4 kittens at each age. The principle response properties studied were direction selectivity, ocular dominance, orientation selectivity, surround inhibition, rate of adaptation to repeated visual stimulation, and response strength and consistency.

The results indicated that the time-course of development differs for different response properties. For example, direction selectivity was present in only 17% of the cells at 2 wks of age and did not reach adult levels (about 80% direction selective) until 8 wks of age. In contrast, the percentage of binocular cells in 2 wk old kittens (81%) already was similar to that of adults. Receptive-field surround inhibition has a developmental time-course similar to that of direction selectivity. Fewer than 5% of the cells had inhibitory receptive-field surrounds at 2 wks of age and the proportion progressively increased to 32% (adult levels) at 8 wks of age. The presence of fast adaptation to repeated visual stimulation, rarely found in adult PMLS cortex, decreased from 73% of the cells at 2 wks to only 10% at 12 wks. Response strength and consistency, while variable between cells at any age, tended to increase during development. Very few orientation selective cells were seen at any age (0 to 10%).

These findings have implications for mechanisms of physiological compensation observed in PMLS cortex of adult animals that had lesions of the ipsilateral visual cortex at early ages. For example, previous studies indicate that direction selectivity in PMLS cortex is lost following visual cortex damage in adult animals but not following damage in 1 day to 12 wk old kittens. Since few PMLS neurons were direction selective in 2 to 4 wk old kittens, the present results suggest that the appearance of this property following lesions at or before 4 wks of age is due to de novo development and not to the maintenance of an already extant property. In contrast, the appearance of normal binocularity following early visual cortex damage (up to 18 wks of age), but not following chronic adult damage, may be due to the maintenance of a property that already had developed at the time of the lesion.

## CELL LINEAGE: DIFFERENTIATION AND DEVELOPMENT II

236.1 THE ORIGIN OF ASTROCYTES IN THE OPTIC NERVE OF THE NEWBORN RAT. R.P. Skoff and R. Sayre*. Dept. of Anatomy, Wayne State University Sch. of Med., Detroit. MI 48201.

The goal of this study was to determine whether astrocytes in the optic nerve of newborn rats arise from cells which already express astroglial specific markers or from an undifferentiated population of cells. To answer this question, glial fibrillary acidic protein (GFAP) immunocytochemistry was combined with thymidine autoradiography usinging the following procedure. 2 and 4 day old rats were perfused with aldehydes one hour following an injection of [3H]- thymidine. The brain and intracranial segment of the optic nerve were cut sagitally with a Vibratome and the sections immunoreacted with GFAP antisera using the PAP procedure. The tissue was then embedded in Araldite, 2µm thick sections were cut and the sections processed for autoradiography.

At 4 days postnatal (dpn), many thymidine labelled cells are also GFAP+ in the optic nerve, chiasm and tract. Some of the thymidine labelled cells are as intensely GFAP+ as any of the GFAP+. non-thymidine cells, indicating that astrocytes rich in GFAP undergo proliferation in normal development. Other thymidine labelled cells are moderately GFAP+ while still others are marginally stained. Because of the gradation in staining, precise quantitation of the number of GFAP+, thymidine labelled cells is difficult but an estimate of the proliferating cells which are GFAP+ in the optic system at this age is 30- 50%. GFAP+, thymidine labelled cells are present at both the periphery of the nerve and in the parenchyma. At 2 dpn, a few thymidine labelled cells are clearly GFAP+. At this age, immunostaining of cells is less intense than at 4 dpn and definitive identification of GFAP+ cells in the semi-thin sections is often difficult. Accordingly. the number of GFAP+, thymidine labelled cells cannot be determined at this time but our impression is that they account for even a larger percentage of the labelled cells than at 4 dpn.

The combined thymidine autoradiography and immunocytochemistry confirm previous electron microscopic and autoradiographic studies showing that astrocytes in the optic nerve proliferate during the first two weeks of postnatal development (Skoff, R.P. et al. J. Comp. Neurol. 169:291. 312, 1976). These cells are properly named astroblasts. Following division, these astroblasts most likely either reenter the cell cycle as astroblasts or exit to form astrocytes. The results of this study show that cell division and cell differentiation are not mutually exclusive processes in the central nervous system (CNS), a finding which is in keeping with the developmental properties of many other non-CNS cell lines. Most importantly, this study demonstrates that most astrocytes generated in the postnatal optic nerve do not arise from an undifferentiated precursor but from cells already committed to the astrocyte lineage. SUPPORTED BY NS15338, NS18883 and NS18898.

236.2 THE DIFFERENTIATION OF RADIAL GLIAL CELLS. K. Frederiksen*, R. McKay; WHITAKER COLLEGE E25-435, M.I.T., Cambridge, MA 02139; S. Hockfield, C.S.H. Lab, P.O. Box 100, Cold Spring Harbor, NY 11724.

In all vertebrates, the cells of the central nervous system are derived from one specialized region of the embryonic ectoderm. After gastrulation, a signal from the underlying mesoderm causes the neural plate to go through a morphogenetic change to form the neural tube. In previous work, we generated reagents which identify the major cell types of the rat neural tube (radial glial cells, neurons, blood vessels). One of these monoclonal antibodies identifies radial glial cells in embryonic day 15 (E15) rat neural tube. The antibody was shown to recognize a 200 Kd polypeptide on immunoblots. It was also shown that this 200 Kd antigen is expressed in many of the cells in the early neural tube before the neurons start to differentiate. Because of the interest in the origin of neurons and glial cells, we have now extended our studies. We have dissected neural tissue from E10 to adult and dissociated the cells with high yield. Immunohistochemistry on these dissociated cells have enabled us to obtain quantitative data on the proportion of cells which express the radial glial differentiation antigen at different developmental stages. This 200 Kd antigen is expressed in 5-30% of the cells at E10. By E11, greater than 98% of the $10^5$ cells in the neural tube express this antigen. At E15, only 50-60% of the $10^7$ cells in the neural tube express the 200 Kd antigen. At P3, there are no antigen positive cells in the spinal cord, while 50-60% of the cells in the cerebellum and nasal lobe still express the antigen. In the adult CNS, there are no antigen positive cells. These results suggest that both neurons and radial glial cells are derived from a population of E11 neuroepithelial cells which all express the 200 Kd antigen, and confirm the immumohistochemical studies which correlate the disappearance of the antigen with the cessation of cell proliferation and/or neuronal migration in the CNS of the rat.

236.3 GLIAL FIBRILLARY ACIDIC PROTEIN IN RADIAL GLIA OF EARLY HUMAN FETAL CEREBRUM, CEREBELLUM AND SPINAL CORD: A LIGHT AND EM IMMUNOPEROXIDASE STUDY. Ben H. Choi, Division of Neuropathology, University of California Irvine, Irvine, CA 92717.

Glial fibrillary acidic protein (GFAP) is widely recognized to be the major protein constituent of glial filaments, which are distinct from other intermediate filaments. It has been demonstrated by us and by others that radial glia in humans and subhuman primates contain GFAP. Although GFAP is highly resistant to formalin fixation and paraffin embedding, the stainability of GFAP can be greatly influenced by delay in fixation and tissue processing. Thus, GFAP immunoreactivity in paraffin sections of routine autopsy brains derived from spontaneous abortions or stillbirths is highly variable and may lead to mistaken conclusions and interpretations.

In order to assess the nature of GFAP immunoreactivity in radial glia of early human CNS, representative sections of cerebrum, cerebellum and spinal cord of 46 embryos and fetuses ranging from 6 to 20 weeks of ovulation age were processed for GFAP immunocytochemistry using vibratome (10 to 30 μm), paraffin (4 to 6 μm), de-eponized one μm and thin sections. All specimens were obtained by hysterotomy or Porro hysterectomy and were fixed within 10 to 20 minutes after the surgery. For EM and vibratome sections, finely minced samples were fixed in 4% glutaraldehyde for 18 to 24 hours then placed in buffer. Paraffin sections were prepared from tissues fixed in toto in Bouin's fluid for 18 to 24 hours then placed into 80% ethanol until sectioned and embedded. The GFAP antisera from three sources (our own, a gift from Dr. L. Eng, and from Dako) were tested and were found to give identical results.

In all three regions studied, GFAP staining was most prominent and consistent in vibratome and 1 μm sections derived from well fixed specimens. In cerebrum and cerebellum, parallel arrays of radially oriented fibers extending from ventricular to leptomeningeal surfaces were strongly positive for GFAP in fetuses of 10 weeks and older. In spinal cord, GFAP staining was equivocal in 6-week-old embryos but became positive by 8 to 9 weeks. Paraffin sections showed patchy and variable GFAP staining. While radial glia demonstrated delicate and thin staining of GFAP, the ependyma and astrocytes showed strong but patchy staining of GFAP in the same sections. The variability of GFAP staining appeared to depend largely on the status of fixation.

The results of this study conclusively demonstrate that radial glia are demonstrable in early human CNS and that they express GFAP at early stages of development. A great caution must be used when interpreting the results of GFAP immunoreactivity in paraffin sections prepared from autopsy brains with delayed or improper fixation.

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236.4 FORSKOLIN INDUCES GROWTH INHIBITION AND DIFFERENTIATION IN CULTURED CELLS FROM RAT BRAIN. O. Kempski*, B. Wroblewska*, H. Kretzschmar*, N. Merkel*, J. Bembry* and M. Spatz (SPON: I. Klatzo). LNNS, NINCDS, NIH, Bethesda, MD 20205

In-vitro studies using cultured brain cells are hampered by the fact that these cells are exposed to high concentrations of growth factors from fetal calf serum (FCS) as well as from artificial media. Therefore they are often in an undifferentiated state and not necessarily representative for a functional cell in-vivo. Previous studies have employed dBcAMP, an analogue of cAMP, to initiate "differentiation" in glial cells (Sensenbrenner M. et al. in: Tissue Culture in Neurobiology, Giacobini et al. eds., Raven Press, New York, 1980). The mode of action of dBcAMP is not clearly defined. Therefore, forskolin which activates the catalytic subunit of adenylate cyclase, was used to study the role of cAMP in the regulation of mitotic activity. Its action on thymidine incorporation and cAMP production was tested in cultured endothelial, smooth muscle, and glial cells from rat brain. GFAP was evaluated as a measure of glial differentiation.

All three cell types showed a forskolin dose-dependent reduction of thymidine incorporation in the presence of FCS. Maximal inhibition was achieved with 100μM forskolin which reduced thymidine incorporation to levels otherwise found in the absence of FCS (75-95% reduction). 5 day exposure of glial cells to forskolin not only caused striking morphological changes similar to those observed after dBcAMP but also led to an increased expression of GFAP which was demonstrated by immunohistochemistry and ELISA. However, the forskolin induced increase of GFAP was lower than that after exposure to dBcAMP. This may be explained by the differences in cAMP levels found after forskolin and dBcAMP. Forskolin (100μM) steeply increased i.c. cAMP (10-20 fold) only during the first hour of exposure in all cell types studied. dBcAMP (.5mM), on the other hand caused cAMP to increase twofold, an increase lasting for more than 24 hours. This phenomenon might be the result of a gradual degradation of dBcAMP to cAMP and butyrate.

These findings emphasize the importance of cAMP in the regulation of mitotic activity. Moreover, the results suggest that forskolin may serve as a tool to initiate differentiation of brain cells in culture.

236.5 INHIBITION OF ADRENERGIC DIFFERENTIATION OF QUAIL NEURAL CREST CELLS BY THE NOREPINEPHRINE UPTAKE INHIBITOR, DESIPRAMINE. M. Sieber-Blum and J. Silverman*. Dept. of Cell Biology and Anatomy, The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Autonomic neurons of the vertebrate embryo are derived from the neural crest. We report here that the in vitro differentiation of quail neural crest cells into adrenergic neurons is inhibited by chronic exposure to the norepinephrine (NE) uptake inhibitor, desipramine. By use of a quantitative in vitro colony assay (Sieber-Blum, M. and Cohen, A.M., Develop. Biol., 80, 96, 1980) we determined that proliferation of "pluripotent" progenitor cells and progenitor cells committed to the neuronal and pigmented cell lineages was not affected by the drug. However, expression of the adrenergic phenotype was markedly depressed. The frequency of colonies containing cells that scored positive for glyoxylic acid/formaldehyde-induced catecholamine fluorescence was reduced by 50% in the presence of 1 μg/ml desipramine and by 70-80% in the presence of 5 μg/ml desipramine. Differentiation of neural crest cells into pigmented cells was not affected by these drug concentrations. When mature cultures were exposed to even higher concentrations (10 μg/ml) of the drug, melanocytes were lysed, but catecholamine histofluorescence in adrenergic neurons was not affected. This indicated that desipramine was interfering with the expression of the adrenergic phenotype rather than causing depletion of intracellular neurotransmitter. Our results thus suggested that the high-affinity NE uptake mechanism may play a role in the differentiation of adrenergic neurons of the peripheral nervous system. Results obtained with pharmacologically active analogs were in agreement with this hypothesis. Mazindol, like desipramine a strong NE uptake inhibitor, blocked adrenergic differentiation as effectively as desipramine. Chlorpromazine, a weak inhibitor, was slightly inhibitory. Citalopram, a potent serotonin uptake inhibitor, did not interfere with the expression of the adrenergic phenotype. The local anesthetic, lidocaine, did not interfere with neuronal differentiation, suggesting that the inhibitory effect of desipramine cannot be attributed to a non-specific membrane effect. Neither desipramine nor any of the analogs affected the frequency of unpigmented, pigmented and mixed colonies. Since desipramine is a widely used antidepressant, a closer examination of its teratogenic potential may be indicated. (Supported by NIH grant HD15311.)

236.6 TRANSIENT NEURONS IMMUNOREACTIVE FOR PEPTIDES IN THE FETAL CAT'S TELENCEPHALON. J.J.M. Chun, M.J. Nakamura* and C.J. Shatz, Dept. of Neurobiology, Stanford U. Sch. of Med., Stanford, CA 94305.

During the development of the mammalian cerebral cortex the white matter is a complex zone consisting not only of afferent and efferent axons, but also of migrating cells en route to the cortical plate. Recently, we have found that in the fetal cat this zone is actually a neuropil that temporarily contains both synapses (Chun & Shatz, Neurosci. Abst. 9:692, 1983) and a special transient class of cell, the subplate cells (Luskin & Shatz, J.Neurosci. 5, 1985). 3H-thymidine studies have shown that these cells are generated exclusively during the one week period (E24-E30) preceding neurogenesis of the cortical plate, and disappear shortly after birth in concert with the synapses. (About 1% of the subplate cells survive into adult life where they reside in the white matter.) In order to learn more about the functional significance of this transient zone during development, we have used a variety of anatomical methods to characterize further the subplate cells.

Immunohistochemical studies show that the subplate cells are very likely to be neurons. All are immunoreactive for MAP-2 (which in the adult is neuron specific: Matus et al., P.N.A.S. 78:3010, 1981). Subsets are also immunoreactive for somatostatin, neuropeptide Y and glutamic acid decarboxylase. Immunoreactive cells were conclusively identified as subplate cells in double-label experiments in which fetuses were first injected with 3H-thymidine at E27,28, then between E50 and birth, cortex was reacted for immunohistochemistry and finally the reacted sections were processed for autoradiography. Results indicate that the population of cells generated simultaneously is heterogeneous with respect to transmitter phenotype. Immunostaining also provided information about the morphology of the subplate cells. In general, cells have elaborate processes radiating out from one or two primary dendrites that are often directed towards the ventricular surface; some of the processes are beaded and may project upwards into the cortical plate or run for some distance in the subjacent white matter. The cells can also be retrogradely labeled with horseradish peroxidase (HRP) injections made locally into the telencephalic white matter. EM examination of the HRP-labeled cells confirms at the ultrastructural level their neuronal identity and reveals that they receive simple synapses upon their dendrites.

These results suggest that the subplate cells are a unique neuronal class that subserves a transient function during the development of the telencephalon. They may serve as temporary synaptic targets for ingrowing afferent systems and might also play a neuromodulatory role in the development of the mammalian cerebral cortex.

Thanks to Drs. R. Vallee and D. Schmechel for their gifts of antisera against MAP-2 and GAD. Supported by NIH grants EY02858 and GM07365.

236.7 ENDOCRINE CELLS OF MOUSE EMBRYONIC PANCREAS EXPRESS NEURONAL TRAITS DURING REGENERATION. G. Teitelman, J. Lee*, T.H. Joh and D.J. Reis. Dept. of Neurology, Cornell Univ. Med. Coll., New York, NY 10021.

We have previously reported that cells transiently expressing tyrosine hydroxylase (TH), the first enzyme of the catecholamine (CA) biosynthetic pathway are present in the pancreas of mouse embryos from prenatal day 11 (E11) to E15 of development. At E12, some of the transient catecholaminergic cells (TC cells) also contained glucagon while at E14.5 some TC cells contained insulin. Pancreatic islet cells do not express TH after E15, even though the cells containing pancreatic hormones persisted throughout life. These findings suggest that the TH cells of embryonic pancreas are the precursors of some or all pancreatic polypeptide-containing cells of adult islets.

In this study we sought to determine whether the ability of pancreatic cells to express TH is restricted to only that population present in the gland during early development in vivo. To test this, the pancreatic rudiment was removed at E11, the gut explanted and a new pancreas was allowed to regenerate from the pancreatic duct. To elucidate whether a new population of TH cells will reappear in the regenerated pancreas (RP) explants were fixed after several days in culture, sectioned and processed for localization of antigens according to the peroxidase-antiperoxidase (PAP) method.

It was found that, after nine days in vitro, the RP contained many glucagon or TH cells but only few insulin or somatostatin cells. This finding indicates that a new population of TH cells, unrelated to those in vivo, developed in the RP. To elucidate whether glucagon cells of the RP, as those in vivo, also contain TH, histological sections of the explants were processed following a combined immunohistochemical-autoradiographic procedure that allows simultaneous visualization of two antigens. Thus, the sections were sequentially incubated with diluted solutions of antibodies to TH, $^{125}$I-donkey antirabbit IgG, antisera to glucagon, goat antirabbit IgG, PAP and 3-3' diaminobenzidine (DAB). The sections were then dried, dipped in Ilford L-4 emulsion (diluted 1:1) and developed after one to two weeks of incubation at 4°C in a light-tight box. It was found that, in the RP, some cells contained only glucagon or TH, as indicated by the presence of either DAB precipitate or silver grains over the cytoplasm, respectively. A third group of cells were doubly labeled by DAB and silver grains and, therefore, contained both TH and glucagon. We conclude that 1) a new population of TH cells appear during neoformation of the mouse embryonic pancreas and 2) some of these TH cells of the RP pancreas also contain glucagon. These findings suggest that, as in vivo, TH cells of RP are precursors of glucagon-containing cells. Moreover, they also suggest that the expression of this neuronal trait is a normal maturational event of differentiating peptide cells of pancreas and, perhaps, of peptidergic neurons of peripheral and central nervous system. (Supported by NIH Grant HL18974 and NSF Grant PCM-8303019.)

236.8 DIFFERENTIAL DISTRIBUTION OF NEURONAL CELL ADHESION MOLECULES DURING HISTOGENESIS OF THE CHICK NERVOUS SYSTEM. J.K. Daniloff, C.-M. Chuong, G. Levi*, and G.M. Edelman. Dept. of Developmental and Molecular Biology, Rockefeller Univ., New York, New York 10021.

The neural cell adhesion molecule, N-CAM, appears early during the development of the primary embryonic axis and in regions where inductive events occur. This molecule undergoes cell surface modulation (Edelman, Ann. Rev. Neurosci., 7:339, 1984) and its conversion from embryonic to adult forms may help to stabilize neural connections (Chuong & Edelman, J. Neurosci., 4:2354, 1984). The neuron-glia cell adhesion molecule, Ng-CAM, mediates the adhesion between developing neurons and glia (Grumet & Edelman, J. Cell Bio., 98:1746, 1984) and initially appears in the ventral neural tube at E4 (Thiery et al., J. Cell Biol., 100:442, 1985).

We compared the distribution and dynamics of the expression of N-CAM and Ng-CAM during histogenesis of the nervous system in developing and adult white Leghorn chickens. The antigens were individually localized by fluorescent immunohistochemistry using polyclonal antibodies to each CAM. The neural regions examined included the spinal cord, cerebellum, optic tectum, retina, forebrain, olfactory bulb, sciatic nerve, and dorsal root ganglion. Each CAM appeared in a characteristic spatial and temporal pattern during cell movement, fiber outgrowth, tract formation, and myelination. Anti-Ng-CAM antibodies labeled extending neurites and post-mitotic cell bodies. A major part of this labeling was correlated with established times of cell migration in spinal cord and cerebellum and was found in regions undergoing neurite extension such as the developing white matter of spinal cord and optic nerve. In the developing CNS, Ng-CAM was more abundant on neurites than cell bodies; in the adult, it was markedly decreased in myelinated tracts, but persisted in unmyelinated regions such as the olfactory bulb. In the PNS (e.g. dorsal root ganglia and sciatic nerves), Ng-CAM appeared early on both cell bodies and neurites, and persisted on both in the adult, even in the presence of myelin. Anti-N-CAM antibodies labeled both cell bodies and neurites in the CNS and PNS. Compared with that of Ng-CAM, the distribution of N-CAM was more uniform throughout development, although the staining intensity was diminished in the adult.

The distribution of both CAMs showed dynamic reversals as the nervous system developed and as a result, the pattern of CAM expression was markedly different in embryos and adults. This difference may reflect changes in the roles of selective adhesion of the two neuronal CAMs at different times of development.

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236.9 A MONOCLONAL ANTIBODY ANALYSIS OF NEURAL DIFFERENTIATION IN THE OLFACTORY NEUROEPITHELIUM. J. Morgan* and J. Hempstead* (SPON: R. Mangano), Roche Inst. of Molec. Biol., Roche Research Center, Nutley, NJ 07110.

The adult olfactory neuroepithelium contains a population of progenitor neuroblasts that retain the capacity to divide and differentiate into mature, functional, olfactory receptor neurons. Removal of one of the paired olfactory bulbs results in the death of mature receptor neurons in the ipsilateral mucosa. Subsequently, through the proliferation and differentiation of neuroblasts the neuroepithelium becomes reconstituted. However, the dynamics of olfactory neuron differentiation is altered when this is compared to the normal contralateral mucosa. These findings are presumed to indicate that the olfactory bulb can modulate the rate of differentiation of olfactory neurons. We have utilized the paradigm of unilateal olfactory bulbectomy to begin to define some of the molecular and biochemical sequelae of differentiation. As probes to investigate these parameters we employed a previously described panel of monoclonal antibodies (J. Neurosci., 1985, 5: 438-449).

Immediately following bulbectomy there ensues the death of olfactory neurons. However, using a monoclonal antibody to sustentacular cells (SUS-1) it may be seen that these glial-like cells are unaffected by surgery. At late times the neuronal complement of the epithelium is reconstituted. However, there are a number of visible alterations. First, a number of monoclonal antibodies (NEU-4, NEU-9) do not stain or show attenuated staining of ipsilateral mucosa. this is in contrast to other clones (NEU-5) that do not distinguish lesioned from unlesioned epithelium. Further, the NEU-9 antibody reveals a neuronal subset in the ipsilateral mucosa. These are believed to be immature cells since the NEU-9 antigen is expressed early in development when compared to the other neuronal antigens detected by the panel of antibodies. Thus the absence of the olfactory bulb appears to have caused a shift in differentiation towards a more immature state as well as the loss of certain antigenic determinants. A further consequence is the production of neuromas in the ipsilateral mucosa. The presence of these structures have led us to investigate the molecular properties of one particular monoclonal antibody (NEU-5) that stains the neuromas intensely, these are described.

236.10 ONTOGENY OF RAT OLFACTORY EPITHELIUM STUDIED WITH MONOCLONAL ANTIBODIES. A.I. Farbman[1], J.I. Morgan*[2], and J.L. Hempstead*[2]. [1]Dept. of Neurobiology and Physiology, Northwestern University, Evanston, IL 60201 and [2]Roche Institute of Molecular Biology, Nutley, NJ 07110.

Monoclonal antibodies can be used as specific probes to study the phenotypic expression of transient or stable molecular species during neuronal development. It may be possible to monitor the stages of differentiation with these probes and to identify those molecules that play significant roles in neuronal function. We have used some antibodies from a panel of monoclonals made from homogenates of adult rat olfactory mucosa (Hempstead & Morgan, 1985, J. Neurosci., 5:438) to monitor the first appearance of certain proteins of olfactory receptor neurons during development. In immunofluorescent studies on histological sections of adult rat olfactory mucosa, Neu-5 antibody was bound primarily to olfactory nerve bundles and only weakly to perikarya. Neu-4 and Neu-9 bound to both perikarya and axons. Sus-1 bound to sustentacular cells in the main olfactory organ exclusively, i.e., it did not bind to homologous cells in the vomeronasal organ. In rat embryos, Neu-5 and Neu-9 were reactive as early as the 15th embryonic day, but Neu-4 was not demonstrable until the 16th (E16). Sus-1 was faintly reactive at E20. These data will be discussed in the context of a detailed ultrastructural study on developing olfactory receptor cells.

In addition to the existing library, we have made new monoclonals to crude membrane fractions of adult and neonatal rat olfactory mucosa, and to total homogenates of fetal mucosa. Screening was done on frozen histological sections of E20 rat fetal heads by immunofluorescent staining. Various antibodies have been found that are specifically immunoreactive to the luminal surface of olfactory epithelium, olfactory supporting cells and Bowman's glands. Other antibodies were reactive only to the luminal surface of respiratory epithelium, and some to both respiratory and olfactory surfaces.

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236.11 REGULATION OF SPECIFIC NEURON TYPE IN FROG RETINA. T.A. Reh, Department of Medical Physiology, The University of Calgary, Calgary, Alberta T2N 4N1, Canada

In most CNS structures, the ratio of differentiated neuronal types is precisely maintained. For example, in the Rana pipiens retina the ratio of ganglion cells to photo receptors to inner nuclear layer cells is 1:3:7.2. Such a precise numerical ratio among the elements could be generated via selective cell production, differentiation or death; however, all of these mechanisms involve some feedback from the tissue to signal that the appropriate number of neurons of a particular class have been generated or maintained. Although the nature of the mechanisms of cell regulation are unknown, I have found that in the frog retina the regulation of density of a particular amacrine cell subclass is critically dependent on the presence of previously differentiated neurons of that type.

There are two monoamine containing cell types in the frog retina: dopamine and serotonin containing amacrine cells. In the present study Rana pipiens tadpoles received unilateral intraocular injections of 6-hydroxydopamine (6-OH-DA). Tadpole retinas were then stained with specific antisera to tyrosine hydroxylase (TH) or serotonin (5-HT) using serial paraffin sections, cryostat sections or whole mounts. The total number of cells in the retinas was counted and their density calculated. 6-OH-DA treatment was effective in eliminating any TH-immunoreactive (TH-IR) cells within 3 days after the injection; however, no change in intensity or cell number was observed when alternate sections were processed for serotonin or substance P immunoreactivity.

In the tadpole, new neurons are generated in a proliferative zone near the ciliary margin. Within one week following the 6-OH-DA treatment, new TH-IR cells begin to appear in this region. By three weeks this area contains numerous TH-IR cells with a density more than three times greater than that in normal retina. This increased staining of TH-IR amacrine cells does not result from a general increase in cell production at the ciliary margin, since the density of 5-HT containing amacrine cells in peripheral retina of 6-OH-DA treated animals does not increase over the control value. Double labelling with $H^3$-thymidine and TH-antiserum shows that all TH-IR cells in these retina were generated after the neurotoxin treatment, indicating that other amacrine cell types do not transdifferentiate into TH-IR amacrines.

In conclusion, 6-OH-DA destroys TH-IR amacrine cells, or irreversibly depletes this enzyme system in these cells. However, the neurotoxin does not destroy the stem cells for these neurons, and proliferation and/or differentiation of TH-IR amacrines is specifically up-regulated following their destruction.

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236.12 **A ZONE OF EXCLUSIVE ROD NEUROGENESIS IN THE TELEOST RETINA**
**Russell D. Fernald and John Scholes*, Inst. Neuro., Univ. of Or., Eugene, Or. and MRC Cell Biophysics, London.**

**It has long been known that teleost fish add new retinal cells of all types concentrically from a primary retinal blastema located around the iris margin of the eye (Müller, Zool. Jb. 63:275, 1952). We have found that this primary retinal blastema produces incomplete, embryonic retinal tissue with a fixed, low ratio of rods to cones. Additional rods are produced by specialized neuroblasts located in the outer nuclear layer (ONL) concentrated in a circular front about 100 μm inside the retinal margin. These neuroblasts move outward from older to newer retinal tissue, dividing to augment the sparse rod population produced by the primary retinal blastema.**

**One micron serial sections were made tangential to the edge of the optic globe, near the retinal margin. At this position, the ONL comprises a single sheet of receptor nuclei in which the rod nuclei are arranged in a square lattice. Each square lattice corresponds to an array of four double cones arranged around a single central cone. More centrally, rod nuclei are packed confluently in a layer more than one deep, vitread to and separate from the cone nuclei. Here, there are between 4 and 10 rods associated with each double cone, depending on the size of the retina, with larger retinae having more rods corresponding to each cone.**

**By serially reconstructing the retinal margin from 1 μm sections, we identified cells which are located in the retinal region where rod density abruptly increases. These are elongated in the plane of the ONL with dispersed patchy chromatin, and are often associated with mitotic figures. Based on these observations, we hypothesized that they were the source of rod photoreceptors.**

**To test this hypothesis, we injected $^3$H-thymidine (30 μCi/gbw) and sacrificed the animals at various times after injection. At 24 h, heavy label was clustered over mitotic figures and these putative precursor cells. After 96 hr, the putative precursors were comparatively weakly labelled, while heavy clusters of grains appeared only over differentiated rod nuclei. After long survival times, only rod nuclei were labelled. From this we conclude that these cells comprise a dividing population of rod precursors. Most likely they constitute a separate lineage which achieves the regulatory feat of maintaining uniform rod density as the eye grows.**

**Supported by the MRC and grants NIH EY 05051 and F06 TW 00832 to RDF.**

## RECEPTOR MODULATION: UP AND DOWN REGULATION II

237.1 **A NOVEL CHEMOSENSORY RECEPTOR FOR γ-AMINOBUTYRIC ACID (GABA) AND GABA-MIMETICS IS SUBJECT TO BOTH UP-REGULATION AND DOWN-REGULATION.**
**H. G. Trapido-Rosenthal* and D. E. Morse* (SPON: S. K. Fisher). Marine Science Institute and Department of Biological Sciences, University of California, Santa Barbara, CA 93106.**

**The free-swimming larvae of the marine gastropod mollusc Haliotis rufescens can be induced to settle from the plankton and metamorphose to the juvenile form by γ-aminobutyric acid (GABA) and stereochemically similar compounds. This induction is mediated by a GABA-receptor that differs in several respects from heretofore characterized types of GABA-receptors. The order of affinities of this receptor for GABA and related compounds is: β-chlorophenyl-GABA (Baclofen) > GABA > muscimol > 3-aminopropane sulfonic acid (3-APS). Induction of settlement by these compounds is positively cooperative, with a Hill coefficient ($n_H$) of approximately 2. The induction of settlement and metamorphosis by these GABA-mimetics can be up-regulated, or facilitated, in a concentration-dependent, stereochemically specific manner, by the L-α,ω-diamino acids, such as L-lysine and L-α,β-diamino propionic acid (L-DAPA). Up-regulation by diamino acids reduces the $n_H$ for inducing GABA-mimetics from 2 to 1, and increases the affinity of the receptor for these inducers (as measured by the apparent dissociation constant) by 30-fold. This up-regulation does not require the simultaneous presence of facilitating and inducing compounds, and the up-regulated state persists for days. Benzodiazepines do not facilitate the interaction of GABA-mimetics with this receptor. The receptor also can be down-regulated, or habituated, by exposing larvae to GABA before they have developed metamorphic competence. The extent of down-regulation is concentration-dependent, and is slowly but completely reversible. Binding of GABA-mimetics to this receptor appears to control an excitatory efflux of chloride ions. These results suggest that up-regulation by DAPA may be caused by a covalent modification of the receptor, converting it to the high-affinity state. They further suggest that down-regulation by GABA may result from internalization or inactivation of the receptor.**

237.2 DECREASED DENSITY OF BENZODIAZEPINE RECEPTORS AFTER CHRONIC ANTIDEPRESSANT TREATMENTS.
B.E. Suranyi-Cadotte, T.V. Dam* and R. Quirion. Douglas Hospital Research Centre, Verdun, Quebec H4H 1R3.

The benzodiazepine (BZ)/GABA system has been extensively implicated in the action of anxiolytic drugs. Anxiolytic effects have also been demonstrated for a number of antidepressant agents. The present study was undertaken to investigate whether an interaction with BZ receptors may be involved in the mechanism of action of antidepressants. Since the therapeutic effects of antidepressants cannot be ascribed to short-term neuroreceptor changes, we have studied the effect of different classes of antidepressants on BZ receptors in rat brain after chronic treatment. In addition, the effect of these agents on β-adrenergic receptors were also assessed to verify antidepressant efficacy.

Male Sprague Dawley rats received twice daily i.p. injections of either saline or 10 mg/kg desipramine, zimelidine, bupropion or adinazolam for 21 days. 24 hours after the last injection, brains were removed and binding assays performed using $^3$H-flunitrazepam and $^3$H-dihydroalprenolol as ligands to identify BZ and β-adrenergic receptors respectively. Chronic treatment with desipramine, zimelidine, bupropion and adinazolam significantly reduced BZ receptor density ($B_{max}$) by 58% to 75% below control values (for all drug treated groups $p < 0.001$ compared to saline group). Chronic treatment with these agents also reduced the number of β-adrenergic receptors, but only by 12% to 22% below control levels.

Although β-adrenergic receptor down-regulation has been previously proposed as a major mechanism involved in antidepressant action, the present results suggest that an interaction with BZ receptor systems may underly, at least in part, the therapeutic effects of antidepressants.

237.3 TRANSCRIPTIONAL REGULATION OF ACETYLCHOLINE RECEPTOR IN CHICKEN SKELETAL MUSCLE AND IN CULTURED CHICK MYOTUBES. B. H. Shieh*, M. Ballivet* and J. Schmidt, Dept. of Biochem., State University of New York at Stony Brook, NY 11794, and Dept. of Biochem., Sciences II, University of Geneva, Geneva, Switzerland.

We have measured levels of mRNA specific for acetylcholine receptor α-subunit in chick skeletal muscle in vivo and in vitro using a genomic DNA probe for an α-subunit segment (amino acyl residues 241 to 314). Total RNA was extracted by the phenol-chloroform procedure and assayed by incubation with an excess of single-stranded $^{32}$P-labeled probe, S1 nuclease digestion, electrophoresis, and autoradiography.

In the calf-musculature of adult chickens, α-subunit message levels were found to be barely detectable. Two days after section of the sciatic nerve a 50fold rise in α-subunit mRNA was observed which reached a peak at three days after denervation and over a period of two weeks gradually declined, to reach near-normal levels after six weeks when signs of functional reinnervation had appeared. The rise in message levels preceded the rise in receptor concentration by over a day.

Cultured chick myotubes produce acetylcholine receptors at a rate comparable to the receptor synthesis in denervated muscle. This synthetic rate increases two- to three-fold in the presence of the sodium channel blocker tetrodotoxin and the calcium channel blocker D600; it is reduced upon addition of veratridine, which activates sodium channels, and ryanodine which activates the sarcoplasmic reticulum. In all cases investigated the level of RNA recognized by the α-subunit probe changed hours before expression of assembled receptor was noticeably affected.

We conclude that electrical activity of plasma membrane controls acetylcholine receptor expression by a transcriptional mechanism.

237.4 THE EFFECTS OF MULTIPLE HIGH DOSES OF METHAMPHETAMINE ON MUSCARINIC CHOLINERGIC RECEPTORS IN THE RAT CNS. R.T. McCabe, G.R. Hanson, J.K. Wamsley, and J.W. Gibb. Departments of Pharmacology, Psychiatry, and Biochemical Pharmacology and Toxicology, University of Utah, Salt Lake City, UT 84132.

Our laboratories and others have demonstrated that multiple high doses of methamphetamine (METH) alter dopamine (DA), serotonin (5-HT), and muscarinic cholinergic nervous systems associated with basal ganglia and mesolimbic structures in the rat. Furthermore, we also have reported a reduction in the number of DA receptors in response to multiple high doses of METH (McCabe et al., Fed. Proc. 44:1829, 1985). Therefore, we investigated the effects of multiple high doses of METH on muscarinic cholinergic receptors paralleled with the METH-induced biochemical alterations in an effort to elucidate the relationship between these receptors and the biochemical events that occur with METH treatment.

Animals were administered METH (15 mg/kg, s.c.) or vehicle every 6 hours for 5 doses. Brains were removed 18 hours following the final dose and coronal tissue slices were obtained. The binding of two muscarinic cholinergic antagonists, $^{3}$H-N-methyl scopolamine (NMS) and $^{3}$H-pirenzipine (PZ), was measured autoradiographically. Both $M_1$ and $M_2$ receptors are labeled with $^{3}$H-NMS (Wamsley et al., Brain Res., 200(1):1, 1980), whereas $^{3}$H-PZ preferentially labels $M_1$ receptors (Wamsley et al., Life Sci., 34:1395, 1984).

The binding properties of muscarinic cholinergic receptors can be characterized on the basis of their affinities for muscarinic cholinergic agonists (Birdsall et al., Mol. Pharmacol., 14:723, 1978). METH treatment elicited a significant reduction in the binding of $^{3}$H-NMS to the high affinity receptors in areas which included the caudate-putamen (33% reduction), nucleus accumbens (24%), olfactory tubercle (40%), superficial layer of the cortex (33%), and CA3 of the hippocampus (24%). The binding of $^{3}$H-NMS to the low affinity receptors also was decreased in the caudate-putamen, nucleus accumbens, and olfactory tubercle of animals treated with METH. Alternatively, the binding of $^{3}$H-PZ to the $M_1$ receptors did not appear to change in response to METH. Thus, a decrease in receptor binding following METH treatment appears to occur exclusively at the $M_2$ receptor sites. The time-course of the METH-induced receptor alterations will be discussed as well as Scatchard analysis of saturation data.

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237.5 DRUG RESPONSIVITY FOLLOWING NERVE INJURY IN THE PINEAL GLAND: A MODEL SYSTEM. K. A. Haak*, D. M. Bronstein, and L. D. Lytle (SPON: G. H. Jacobs). Laboratory of Psychopharmacology, Department of Psychology, University of California, Santa Barbara, CA 93106.

Activity of the pineal gland enzyme, N-acetyltransferase (NAT) is controlled by the release of norepinephrine from sympathetic nerves onto postsynaptic pinealocyte β-noradrenoceptors. Receptor stimulation activates adenylate cyclase, leading to increased intracellular concentrations of cyclic AMP and enhanced NAT activity via a protein kinase mechanism. Denervation of the postganglionic sympathetic fibers arising from the superior cervical ganglia results in the phenomenon of denervation receptor supersensitivity, which involves an increased responsiveness of postsynaptic pinealocyte neurochemical mechanisms as a result of the loss of neurotransmitter input. Isoproterenol and other β receptor agonist drugs cause supersensitive-like responses in NAT activity following denervation (see Axelrod, Science, 184:1341, 1974).

In the present experiments we compared time dependent alterations in the actions of drugs thought to depend on noradrenergic presynaptic, pinealocyte β-noradrenoceptor, or pinealocyte intracellular, receptor-linked mechanisms following superior cervical ganglionectomy (SCGX) in rats. Male Sprague-Dawley albino rats weighing 300-400 g were anesthetized with sodium pentobarbital and subjected to bilateral SCGX. Following recovery from surgery rats were given ad libitum access to food and water and exposed to a 12:12 hr light:dark cycle (lights on at 0700 hr). Pineal glands were removed from intact and SCGX animals 1 or 7 days following surgery, then incubated in $10^{-3}$ M HCl vehicle, desmethylimipramine (DMI, $10^{-4}$ M) a presynaptic reuptake inhibitor, isoproterenol ($10^{-5}$ M) or isobutylmethylxanthine (IBMX, $10^{-4}$ M) a drug which inhibits phosphodiesterase catalyzed degradation of intracellular cAMP. The DMI-induced increase in pineal gland NAT activity in intact animals was attenuated in SCGX animals at 1 and 7 days postsurgery. Both isoproterenol and IBMX caused significant increases in NAT activity in intact animals and the enzyme induction caused by these drugs was potentiated in SCGX animals at 1 and 7 days.

These data show that denervation of the pineal gland attenuates the action of drugs whose effects are mediated by presynaptic mechanisms but potentiate the action of drugs acting directly on the receptor or on postreceptor intracellular biochemical mechanisms. (Supported by grant MH-31134).

237.6 $GM_1$ GANGLIOSIDE TREATMENT COUNTERACTS AND NORMALIZES DOPAMINERGIC RECEPTOR SUPERSENSITIVITY INDUCED BY NEUROLEPTICS. A. Leon, L. Cavicchioli*, S. Calzolari*, A. Consolazione* and G. Toffano. Fidia Neurobiological Research Laboratories, Abano Terme, Italy

Striatal dopaminergic (DA) receptor supersensitivity is known to occur not only following chronic treatment with DA antagonists but also following chemical injury of the nigro-striatal pathway. In this context studies from our own and other laboratories have recently documented that chronic $GM_1$ monosialoganglioside treatment counteracts the development of striatal DA receptor supersensitivity in adult rats chronically treated with haloperidol (Agnati, L.F., et al., Neurosci. Letters., 40:293-297, 1983) but not following presynaptic chemical denervation induced by a single unilateral injection of 6-OH-DA directly in the substantia nigra (Toffano G., et al., Acta Physiol. Scand., 122:313-321, 1984).

We now report that chronic $GM_1$ treatment is also capable of facilitating the recovery of receptor normosensitivity in adult rats following haloperidol withdrawal. In particular rats were first treated with haloperidol (2 mg/kg/day i.p.) for 14 days and then, after 5 days of washout, treated with saline or $GM_1$ (30 mg/kg/day i.p.) for 7 or 14 days. Treatment with haloperidol for 14 days produced approximately a 40% increase of the apparent Bmax of striatal $^{3}$H-spiperone binding sites. No modification of the apparent Kd was observed. Post-treatment with $GM_1$ significantly decreased, with respect to saline injection, the apparent Bmax of $^{3}$H-spiperone binding already after 7 days of treatment. After 14 days, the haloperidol-induced receptor supersensitivity completely disappeared in $GM_1$-treated but not in saline-treated animals. Similar $GM_1$ effects were observed following chronic treatment with perphenazine. In addition, $GM_1$ alone did not produce any significant change of $^{3}$H-spiperone binding when administered alone in vivo or after addition in vitro.

The above data indicate that $GM_1$ can effectively not only counteract but also normalize dopamine receptor supersensitivity induced both by butyrophenones and phenothiazines. The mechanism by which $GM_1$ produces this effect is still unknown. Possible effects on striatal dopamine presynaptic activity and/or on neuropeptides which may modulate the dopamine receptor function are currently under investigation.

237.7 ELEVATION OF STRIATAL DOPAMINE RECEPTOR DENSITY AFTER CHRONIC TREATMENT WITH HALOPERIDOL, DOMPERIDONE, OR SULPIRIDE AND ITS PREVENTION FOLLOWING PROLACTIN DEPLETION BY HYPOPHYSECTOMY OR BY CHRONIC TREATMENT WITH CYSTEAMINE. R.E. Hruska, Department of Biochemical Pharmacology, SUNY-Buffalo, Buffalo, NY 14260.

Recently, a role for pituitary hormones in the regulation of striatal dopamine (DA) receptors has been proposed. In most cases, removal of all pituitary hormones by hypophysectomy (Hypox) did not produce a major alteration in the density or the affinity of the striatal DA receptors. However, Hypox altered the increase in density observed after chronic haloperidol (HAL) treatment. When the daily doses of HAL were small (1 mg/kg), Hypox completely prevented the increase in density. When the daily doses of HAL were large (4 or 5 mg/kg), Hypox attenuated the increase in density (Eur. J. Pharm. 76:31 and 85:201). It has been proposed that this change is mediated by the pituitary hormone, prolactin (Life Sci. 30:547). In contrast, chronic treatment with domperidone (DOM) or sulpiride (SUL) elevated serum prolactin levels without altering the density of the striatal DA receptors (Eur. J. Pharm. 93:195).

These studies were designed to readdress the effects of large doses of DOM and SUL, and to use two differentially selective methods for prolactin depletion -- Hypox and chronic cysteamine treatment. Hypox removes all pituitary hormones, while cysteamine treatment depletes prolactin and somatostatin, induces ulceration, and causes depilitation. HAL (1 mg/kg), DOM (1 or 5 mg/kg), and SUL (30 or 100 mg/kg) were administered daily, i.p., for 20 days followed by a 7 day drug-free period. Cysteamine treatment (90 mg/kg, s.c.) preceded each HAL treatment by 2 hours. One hour after the last drug dose, a blood sample was removed from the tail and serum prolactin levels measured by RIA. The densities and affinities of the striatal DA receptors were measured with [$^3$H]spiperone after the 7 day drug-free period.

Chronic treatment with HAL, DOM, or SUL elevated serum prolactin levels and produced an increase in the density of the striatal DA receptors. Hypox prevented the increase in serum prolactin levels and the increase in DA receptor density after these treatments. Cysteamine treatment decreased serum prolactin levels without altering the striatal DA receptor density. Cysteamine treatment also prevented the increase in serum prolactin levels and the increase in density of the striatal DA receptors normally seen after HAL treatment.

These results support the hypothesis that prolactin may exert a modulatory function in the regulation of striatal DA receptor density. This function is likely to be complex since it is only associated with increases in DA receptor density. In addition, the magnitude, duration, and persistence of prolactin elevation required to induce these changes are not known. (Supported in part by MH-38017 and The Research Foundation of SUNY.)

237.8 DOES EXTRACELLULAR COPPER MODULATE PROSTAGLANDIN $E_2$ ($PGE_2$) RECEPTOR ON HYPOTHALAMIC LHRH NEURONS?

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$PGE_2$ stimulates the release of LHRH (luteinizing hormone releasing hormone) from LHRH neuronal terminals. Disulfide bonds have been implicated in $PGE_2$ binding to its receptor and in $PGE_2$ action, and copper can lead to the formation of such disulfide bonds. Copper has been found in high concentrations in hypothalamic tissue, particularly in axonal terminals. We have hypothesized that copper is present in high concentrations in the extracellular space of LHRH neurons, as a consequence of copper release from copper-rich axonal terminals. Such extracellular copper could facilitate $PGE_2$ action by inducing the formation of disulfide bonds on the receptor itself or on other related molecules that affect $PGE_2$ binding/action. In this study, we addressed the question: Does extracellular copper facilitate $PGE_2$ stimulation of LHRH release from median eminence (ME) explants? ME of male rats were incubated for 5 min with 100 µM copper (in the form of CuHistidine; CuHis) and then for 15 min with 50 µM $PGE_2$ ($Cu/PGE_2$). Release of LHRH during the latter 15-min period was quantified by RIA. Controls were incubated with CuHis or $PGE_2$ alone. Although CuHis and $PGE_2$ each stimulated LHRH release about 2-fold over the basal release (net stimulated release being 3.3 and 2.2 pg/5min per ME, respectively), $Cu/PGE_2$-stimulated release (12.5 pg/5 min per ME) was two times greater ($P < 0.001$) than the sum of CuHis-and $PGE_2$-stimulated release. Thus, copper facilitates $PGE_2$ action. The facilitatory effect of copper was dependent on the dose of copper and of $PGE_2$, and it was demonstrable only if $PGE_2$ was added immediately after CuHis but not 15 min before or 15 min after CuHis. By comparison to $PGE_2$, $PGD_2$ was much less effective in stimulating LHRH release and copper did not facilitate $PGD_2$ action. These results are consistent with a short-lived, $PGE_2$-specific effect of copper on the binding properties of the $PGE_2$ receptor or on the functional state of an early (maybe initial) post-receptor event involved in the process of $PGE_2$ stimulation of LHRH release from LHRH neuronal terminals.

237.9 CENTRAL NEUROTRANSMITTER RECEPTOR CHANGES FOLLOWING CHRONIC EXPOSURE TO LOW LEVELS OF LEAD. J. Offermeier, J.M. van Rooyen and J. Rossouw. Dept. of Pharmacology and MRC Research Unit for the Design of Catecholaminergic Drugs, Potchefstroom University, Potchefstroom 2520, South Africa.

Three groups of Wistar rats were exposed to low concentrations of lead at different stages of their development for periods of 21-23 days. Exposure was initiated at conception (group 1), at parturition (group 2) or after weaning had occurred (group 3). Blood levels of lead achieved during exposure in all 3 groups did not exceed 75µg/dℓ.

When a mass of 160 ± 10g was achieved the rats were sacrificed and radioligand binding studies were performed. The ligands $^3$H-di-OH-alprenolol (for estimation of receptor numbers and receptor affinity of β-adrenoceptors), $^3$H-clonidine (for $\alpha_2$-adrenoceptors), $^3$H-serotonin (for $S_1$ serotonin receptors), $^3$H-ketanserin (for $S_2$ serotonin receptors), $^3$H-naloxone (for opioid receptors) and $^3$H-dopamine for (D2 dopamine receptors) were used.

Lead exposure results in increases in the number of cortical β-adrenoceptors in group 2; the numbers of cortical $\alpha_2$-adrenoceptors in group 1, 2 and 3, the numbers of hippocampal $S_1$ receptors in groups 1 and 2 and the number of striatal D2 receptors in group 2. The number of forebrain opioid receptors in group 3 is decreased by lead exposure. No change in the numbers of cortical $S_2$ receptors results from lead exposure in any of the groups. The affinity of the $^3$H-ligand for hippocampal $S_1$ receptors is significantly decreased while the affinity of the $^3$H-ligand for forebrain opioid receptors is significantly increased in groups 2 and 3 resp.

The results show that exposure to low levels of lead does not only change the receptor numbers but may also result in affinity changes at various central neurotransmitter receptors. The developmental stage at which lead exposure occurs appears to be critical, as manifested by the findings that the changes which occurred in the 3 groups of rats were not identical.

237.10 ELECTROCONVULSIVE SHOCK SELECTIVELY DOWN-REGULATES BETA-1-ADRENERGIC RECEPTORS IN SPECIFIC AREAS OF RAT BRAIN. K.J. Kellar[1], C.A. Stockmeier[1], T.C. Rainbow[2] and B.B. Wolfe[2]. Departments of Pharmacology,[1] Georgetown University School of Medicine, Washington DC 20007, and [2]Medical School, University of Pennsylvania, Philadelphia, PA 19104.

Repeated administration of electroconvulsive shock (ECS) or antidepressant drugs decreases the number of beta-adrenergic receptors in certain areas of rat brain. Ligand binding studies in homogenates have shown that ECS (once daily for 7 to 12 days) decreases these receptors in the cerebral cortex and hippocampus, but not in the striatum, cerebellum or hypothalamus. The pattern of decrease suggested that ECS selectively decreases beta-1-adrenergic receptors, and only in those brain areas in which ECS activates norepinephrine axons (Kellar et al., J. Neurochem. 37, 1981).

Quantitative autoradiography of $^{125}$I-iodopindolol ($^{125}$Ip) binding in the presence of a selective beta-2-adrenergic antagonist (ICI 118,551) or a selective beta-1-adrenergic antagonist (ICI 89,406) has provided a detailed analysis of the anatomical distributions of beta-1- and beta-2-adrenergic receptors, respectively, in rat brain (Rainbow et al., PNAS 81, 1984). We have now measured the effects of repeated ECS (12 days) on beta-1- and beta-2-adrenergic receptors in rat brain using this selective quantitative autoradiographic method. Specific binding was defined as the difference in $^{125}$Ip bound in the absence and presence of 50 uM isoproterenol.

Analyses of the autoradiograms indicated that ECS decreases the binding of $^{125}$Ip in the cerebral cortex, hippocampus, amygdala and thalamus. In each of these areas, only binding to beta-1-adrenergic receptors was decreased. Binding to beta-2-adrenergic receptors was unaffected. Binding of $^{125}$Ip in other areas of brain analyzed to date, including the striatum, cerebellum and hypothalamus, appeared to be unaffected by ECS.

This autoradiographic analysis has revealed selective effects of ECS on beta-1-adrenergic receptors in specific layers of the cerebral cortex, regions of the hippocampus and nuclei of the amygdala and the thalamus. Localization of the effect of ECS on beta-1-adrenergic receptors may aid in determining the anatomical structures involved in antidepressant mechanisms.

237.11 CORTICOSTERONE DECREASES MUSCARINIC, NORADRENERGIC AND GLUTAMATE RECEPTORS IN RAT HIPPOCAMPUS. S. Halpain, B. Nock, G.P. Dohanich, and B.S. McEwen. The Rockefeller University, New York, New York

Steroid hormones regulate a variety of cellular responses, including aspects of synaptic neurotransmission. Studies from several laboratories have shown that steroids can regulate neurotransmitter receptors in neuronal and peripheral tissues. We therefore tested the ability of corticosterone (CORT) to regulate neurotransmitter receptors in the rat brain. Previous work from this laboratory indicated that CORT might regulate certain receptors in hippocampus (Biegon et al, 1985; Brain Res. 332:309).

In the first experiment, male rats were adrenalectomized (ADX) or sham-operated (SHAM). After 2 days one-half the ADX animlas were given 300mg pure CORT in subcutaneous pellets for 5 days; these were supplemented on day 5 with 200mg additional CORT pellet. After 10 days, all three groups were sacrificed and neurotransmitter receptor binding was assessed by standard in vitro autoradiographic methods. Muscarinic receptors were labeled with 1 nM $^{3}$H-N-methylscopolamine (NMS), $\alpha_2$-adrenergic receptors with 2.5nM $^{3}$H-p-aminoclonidine (pAC) and glutamate receptors with 300nM $^{3}$H-glutamate. Binding in various brain areas was measured densitometrically. Corticosterone treatment had a similar effect in decreasing binding for all 3 ligands. CORT-treated animals showed 15-30% lower binding than ADX controls. The SHAM group was either somewhat lower or not significantly different than ADX. This effect was seen in the hippocampal formation ($^{3}$H-NMS and $^{3}$H-glutamate) and in both the hippocampus and lateral septum ($^{3}$H-pAC), two brain areas which highly concentrate adrenal steroids. The effect was not observed in other brain areas measured, including medial septum, striatum, cortex, and areas of the thalamus and hypothalamus. The effect was most pronounced in regions of the dorsal hippocampus; it was either much smaller or not observed at all in regions of ventral hippocampus.

In a second series of experiments we sought to determine the nature of the binding change. Scatchard analysis of $^{3}$H-NMS and $^{3}$H-pAC binding to homogenates of dorsal hippocampus indicated that the effect of CORT was to decrease the maximal number of sites without affecting their affinity for the ligand.

A third study utilized daily injections of CORT instead of implants of CORT pellets in order to more closely mimic physiological exposure to the hormone. Membranes prepared from dorsal hippocampus showed significantly lower $^{3}$H-glutamate binding in CORT-treated ADX rats (10mg daily for 5 days) than in ADX rats given no hormone replacement. Moreover, this change apparently occurs without specificity to a subtype of glutamate receptor, since the ability of chloride to stimulate or quisqualate to compete for $^{3}$H-glutamate binding was not altered. These results suggest that the effect of CORT on neurotransmitter receptor number might be a generalized phenomenon, occuring with many receptor types, but that it is specific to brain areas which selectively bind adrenal steroids.

## CYCLIC NUCLEOTIDES I

238.1 GASTROINTESTINAL MESSENGER SUBSTANCES MODULATE EXCITABILITY IN MYENTERIC PLEXUS NEURONS THROUGH ACTIVATION OF ADENYLATE CYCLASE. J.D. Wood, D.H. Zafirov and J.M. Palmer. Dept. of Physiol., School of Medicine, University of Nevada, Reno, Nevada 89557.

Slow synaptic excitation (slow EPSP) in myenteric ganglion cells can be mimicked by application of several different peptides and biogenic amines. Many of these substances have been linked with activation of adenylate cyclase in other types of neurons and neural tissues. The aim of our investigation was to identify gastrointestinal messenger substances which exert their actions through adenylate cyclase to influence the electrical behavior of guinea-pig AH/type 2 myenteric neurons. Conventional intracellular methods with 3 M KCl-filled microelectrodes were used to record and inject electrical current in neurons from myenteric plexus preparations superfused with carboxygenated Krebs solution in vitro. Putative messenger substances were applied either in the superfusion solution or by pressure ejection from micropipettes. The diterpene, forskolin, a potent activator of adenylate cyclase, mimicked the slow EPSP in myenteric neurons when applied in the superfusion solution (0.05-50 uM) or by pressure microejection (50 uM). Pretreatment with adenosine or 2', 5'-dideoxyadenosine (10-100 uM) applied in the superfusion solution suppressed or abolished the action of forskolin. This suggested that inhibitory purinergic receptors were linked to adenylate cyclase in these neurons, specifically adenosine-$A_1$ receptors, that could prevent intraneuronal accumulation of cyclic AMP induced by stimulatory messengers. Therefore, we reasoned that pretreatment with adenosine or adenosine analogs with high affinity for $A_1$ receptors should offset the electrophysiological effects of excitatory messenger substances that stimulate adenylate cyclase. Vasoactive intestinal peptide (VIP), gastrin-releasing peptide (GRP), bombesin, cholecystokinin-octapeptide (CCK-OP), caerulein, substance P, histamine and serotonin each mimicked the slow EPSP when applied either in the superfusion solution or by pressure microejection. The effects of these substances occurred after blockade of axonal spike generation and synaptic transmission in the presence of tetrodotoxin (1-2 uM), and were potentiated in the presence of 0.1 uM forskolin. Pretreatment with adenosine or adenosine analogs prevented the actions of VIP, GRP, bombesin, CCK-OP, caerulein and histamine. However, the actions of substance P and serotonin could not be blocked with adenosine in the same cells where it had successfully blocked the other substances. Results suggest that VIP, GRP, bombesin, CCK-OP, caerulein and histamine act through adenylate cyclase to effect long-lasting modulation of excitability in AH/type 2 myenteric plexus neurons. Substance P and serotonin exert similar effects on neuronal excitability, but they may do so through mechanisms independent of the cyclic AMP generating system. (Supported by NIH Grant RO1 AM 26742).

238.2 SECOND MESSENGER FUNCTION OF CYCLIC AMP MEDIATES MODULATION OF EXCITABILITY IN MYENTERIC PLEXUS NEURONS. J.M. Palmer, D.H. Zafirov, P.R. Nemeth and J.D. Wood (SPON: C. Ort). Dept. of Physiol., Univ. of Nevada, Reno, NV 89557.

Synaptic input to myenteric neurons of guinea-pig small intestine generates a slow synaptic mechanism of excitation associated with prolonged membrane depolarization and enhanced somal excitability involving ionic conductance changes. This suggests that generation and accumulation of an intraneuronal second messenger might play a significant role in long-term modulation of neuronal excitability. We hypothesized that intrasomal cyclic AMP elevated by putative messenger substances could be the common intermediate step in generation of slow synaptic excitation (slow EPSP) in AH/type 2 myenteric neurons. Conventional intracellular methods were used to record electrical behavior from ganglion cells of myenteric plexus preparations superfused with carboxygenated Krebs solution in vitro. Forskolin, a potent activator of adenylate cyclase, 8-(4-chlorophenylthio) cyclic AMP and 8-bromo cyclic AMP, membrane-permeable analogs of cyclic AMP, and the phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine (IBMX), were applied in the superfusion solution. Cyclic AMP, 5'AMP and cyclic GMP were iontophoresed into the cell body through the intracellular recording electrode with anodal current pulses. Microelectrodes used for iontophoresis were filled with 0.9 M KCl plus 0.2 M sodium salt of the compounds to be injected and had resistances of 80 to 140 Mohm. Extracellular application of forskolin (0.05-50 uM), 8-(4-chlorophenylthio-) and 8-bromo cAMP (10-100 uM), IBMX (10-100 uM) or intracellular iontophoresis of cyclic AMP simulated alterations of neuronal electrical behavior characteristic of the onset of the slow EPSP. This was manifested as: 1) long-lasting membrane depolarization; 2) enhanced somal excitability with spontaneous and increased stimulus-evoked action potential discharge; 3) anodal-break excitation; 4) decreased membrane ionic conductance; and 5) suppression of post-spike hyperpolarizing potentials. Depolarization during cyclic AMP-induced excitation showed a reversal potential for this effect close to the potassium equilibrium potential. The actions of forskolin and the cyclic AMP analogs were potentiated by IBMX, and occurred after blockade of axonal spike generation and synaptic transmission in the presence of tetrodotoxin (1-2 uM). Intracellular iontophoresis of 5'AMP and cyclic GMP did not affect the electrical behavior of myenteric neurons. Results suggest that the biochemical basis for the slow EPSP myenteric in AH/type 2 neurons is initiated by coupling of membrane receptors for "first messenger" substances with adenylate cyclase in the somal membrane. The ligand-receptor-enzyme interaction then triggers synthesis and accumulation of cyclic AMP which acts as the intraneuronal "second messenger" mediating long-term modulation of somal excitability involved in integrative functioning of the enteric nervous system. (Supported by NIH Grant RO1 AM 26742).

283.3 RECEPTOR-MEDIATED CYCLIC NUCLEOTIDE REGULATION OF IMPULSE FREQUENCY IN PERIPHERAL NERVE. M-Y.Lo* and J.J.Kendig. Dept. of Anesthesia, Stanford Univ. Sch. of Med., Stanford, CA 94305

We have previously demonstrated that refractory period in peripheral nerve is subject to modulation by cyclic AMP (cAMP), the endogenous content of which is increased by β-adrenergic receptor stimulation. Both β-agonists and a lipid soluble cAMP analogue, dibutyryl cyclic AMP (db-cAMP), increase the refractory period and thus decrease the maximum frequency at which the nerve can carry action potentials. We now report an opposing regulation by cyclic GMP (cGMP), possibly mediated by a cholinergic receptor.

Sciatic nerves were dissected from adult frogs (Xenopus laevis), desheathed and mounted in a chamber permitting continuous perfusion of a central 1 cm length of the nerve. The proximal end was laid over stimulating electrodes in a pool of mineral oil. Stimuli were adjusted to be well supramaximal. Recording electrodes were placed in the central perfusion compartment and in a distal pool filled with 140 mM KCl to provide monopolar recording. Drugs were dissolved in frog Ringer's solution buffered with 10 mM HEPES to a pH of 7.4. All experiments were carried out at room temperature. Refractory period was assayed by determining the amplitude of the second of a pair of impulses relative to the first.

A lipid-soluble analogue of cGMP, dibutyryl cGMP (db-cGMP), decreased the refractory period at concentrations of 1 - 2 mM. The effect was discernable 15 - 20 minutes following application, and maximal at 30 minutes. Increase in relative amplitude of the second pulse over controls was confined to interstimulus intervals of 1.0 - 1.2 msec; there was no apparent effect at intervals 1.5 msec or longer. Control experiments showed no decrease in refractory period on switching from one drug-free solution to another over the same time period. The effect of db-cGMP was mimicked by the cholinergic agonist carbamylcholine, $2x10^{-5}$M; onset time of the effect was approximately the same as for db-cGMP, but the maximum effect was achieved later, at 40 minutes. Neither agent appeared to affect the amplitude of singly evoked compound action potentials, but both slightly increased their width at half maximal amplitude.

Both cAMP and cGMP are endogenous in peripheral nerves. The responses to db-cGMP and carbamylcholine suggest that in this tissue some neuronal function which influences refractory period is subject to modulation by cholinergic stimulation of cGMP, in a direction opposite to the modulation produced by β-adrenergic stimulation of cAMP.

238. FORSKOLIN, ACTING AT A SITE DISTAL TO CALCIUM ENTRY, ENHANCES THE SECRETORY RESPONSE OF A PITUITARY TUMOUR CELL. S. Guild*, J.W. Kebabian*, and E.A. Frey. Experimental Therapeutics Branch, NINCDS, NIH, Bethesda, MD 20205.

Mechanisms, other than an acute increase in the concentration of cytosolic calcium ($[Ca_i]$), can regulate the physiological process of exocytosis (Baker, P.F. Nature., 310: 629-630, 1984). Cyclic AMP (cAMP) represents one metabolic stimulant of exocytosis. One effect of cAMP upon secretion, the potentiation of stimulated release, was studied in the prolactin-secreting 7315c rat pituitary tumour cell line. We enhanced cAMP synthesis with forskolin (Seamon, K.B. & Daly, J.W. J. Cyclic. Nucleotide. Res., 7: 201-224, 1981). We raised the $[Ca_i]$ with either a potassium challenge, believed to transiently open voltage-dependent calcium channels (Tomiko, S.A., Taraskevich, P.S. & Douglas, W.W. Neurosci., 6:2259-2265, 1981) or ionomycin, a calcium ionophore (Liu, C.M. & Hermann, T.E. J. Biol. Chem., 253: 5892-5894. 1978).

The basal rate of release of prolactin from this tumour was 3.0 ± 0.2 ng prolactin/$10^6$cells/5 mins (mean ± SEM, n=12). A 5 min challenge with potassium (60 mM) increased the rate of prolactin secretion by 3-fold. Treatment of the cells, for 5 mins with forskolin (10 μM), caused only a 0.5-fold increase in the rate of hormone release. However, following such a treatment with forskolin, the potassium-evoked secretory response was doubled. Using the Quin 2 fluorescent dye technique (Tsien, R.Y., Pozzan, T. & Rink, T.J. J. Cell. Biol., 94: 325-334. 1982), the $[Ca_i]$ within 7315c cells was determined to be 107 ± 2 nM (mean ± SEM, n=46). Potassium depolarisation elicited a transient 3-fold increase in $[Ca_i]$. Forskolin did not alter $[Ca_i]$ or the potassium-evoked rise in this parameter. Ionomycin elicited a 4-fold enhancement of prolactin release. Forskolin doubled the ionomycin-evoked release of prolactin. Ionomycin elicited a 7.5-fold increase in $[Ca_i]$, which was unaffected by the pretreatment with forskolin.

These data demonstrate that the secretory response of the 7315c cells, elicited by a fixed increase in $[Ca_i]$, is variable. Forskolin, and by inference cAMP, can increase the amount of hormone released, in response to either potassium or ionomycin, without affecting the stimulated increase in the $[Ca_i]$. We hypothesise that the effect of forskolin is at a site different from, and distal to, the voltage-dependent calcium channel. cAMP is acting upon the capacity of the cell to respond to a fixed increment in $[Ca_i]$.

238.4 STIMULATION OF cGMP SYNTHESIS BY BAY-K-8644 IN MOUSE AtT-20 PITUITARY TUMOR CELLS. S. Heisler* (SPON: L. Larochelle). Unité Bioregulation Cellulaire, Centre hospitalier de l'Université Laval, Québec, Canada, G1V 4G2.

In mouse AtT-20/D16-16 (AtT-20) pituitary tumor cells, BAY-K-8644, depending on its concentration stimulates and antagonizes ACTH secretion. This effect presumably is due to a similar concentration dependent effect on calcium entry in these cells. Because the cytoplasmic enzyme guanylate cyclase is activated by calcium entry in several cells systems, it was of interest to determine whether BAY-K-8644 also had a biphasic effect on the intracellular accumulation of cGMP in AtT-20 cells. It was observed that at concentrations of $10^{-10}$ to $10^{-6}$M, BAY-K-8644 had no effect on basal cGMP levels; however, it did stimulate formation of the nucleotide at concentrations ($10^{-6}$ to $10^{-4}$ M) which were associated with its ability to antagonize basal and (-) isoproterenol-stimulated ACTH secretion. Neither the cationophore A-23187, nor depolarizing concentrations of $K^+$ mimicked the effect of BAY-K-8644 on cGMP formation; similarly, other calcium channel antagonists such as nifedepine or verapamil (up to $10^{-4}$M) were without effect on cyclic nucleotide synthesis though both did inhibit agonist stimulated ACTH secretion. To further test whether cGMP formation was correlated with decreased secretory responsiveness, the effects of known stimulators of guanylate cyclase activity, sodium nitroprusside and sodium azide were assessed on cGMP formation and ACTH secretion. Both sodium nitroprusside and sodium azide in a concentration-dependent fashion, increased cGMP accumulation and both compounds inhibited basal and (-) isoproterenol-stimulated ACTH secretion though to a lesser extent than BAY-K-8644. Conclusions: BAY-K-8644 stimulates cGMP synthesis by binding to sites inaccessible or not activated by other calcium channel antagonists (e.g. low affinity sites). Stimulation of guanylate cyclase in AtT-20 cells is independent of inward calcium current i.e. BAY-K-8644 may stimulate a particulate enzyme. Intracellular cGMP may have a negative, intracellular effect on the ACTH secretory process.
(Supported by MRC of Canada)

238.6 PROTEIN KINASES IN THE SUPERIOR CERVICAL GANGLION. A.L. Cahill* and R.L. Perlman, Department of Physiology & Biophysics, University of Illinois College of Medicine, Chicago, IL 60680

To understand the role of protein phosphorylation in the control of neuronal function it is necessary to characterize the protein kinases in neuronal tissue, to identify the endogenous protein substrates for these kinases, and to determine the effects of phosphorylation on the activities of these proteins. In this study we have characterized the protein kinase activities in extracts of the superior cervical ganglion of the rat. The ganglion contained cAMP-dependent, cGMP-dependent, $Ca^{2+}$/calmodulin-dependent, and $Ca^{2+}$/phospholipid-dependent protein kinases. All of these kinase activities were largely in the soluble fraction. A clear dependence of the $Ca^{2+}$/phospholipid-dependent protein kinase on exogenous phospholipids could only be demonstrated in partially purified preparations from which endogenous phospholipids had been removed. The ganglion also contained endogenous substrates that were phosphorylated by the cAMP-dependent, the $Ca^{2+}$/calmodulin-dependent, and the $Ca^{2+}$/phospholipid-dependent protein kinases in vitro. These three kinases appear to phosphorylate distinct but overlapping sets of ganglionic proteins. One endogenous substrate was tyrosine hydroxylase, which was phosphorylated by all three of these kinases. Another endogenous substrate was an acidic 83,000 $M_r$ protein that has been identified as the "87k" acidic synaptosomal protein (Wu et al., PNAS 79,5249,1982) and that appears to be a specific substrate for the $Ca^{2+}$/phospholipid-dependent protein kinase.

Electrical stimulation and treatment with various pharmacological agents increases the phosphorylation of a number of proteins in the intact ganglion. We have begun to investigate which stimuli activate each of the protein kinases by determining which stimuli activate the cAMP-dependent protein kinase. In situ activation of cAMP-dependent protein kinases can be assessed by measuring the "activity ratio" of the kinase in extracts of the ganglion. The "activity ratio" is defined as the kinase activity in the absence of cAMP divided by the kinase activity in the presence of cAMP. Electrical stimulation (20 Hz,5 min), 100 μM forskolin, and 2 μM vasoactive intestinal peptide all increased the "activity ratio" of cAMP-dependent protein kinase in the ganglion. Activation of the cAMP-dependent protein kinase may mediate some of the effects of these agents on protein phosphorylation. Nicotinic and muscarinic antagonists did not block the effect of electrical stimulation suggesting that release of a neurotransmitter other than acetylcholine may be responsible for the activation of the cAMP-dependent protein kinase. (This research was supported in part by grant HL 29025 from NIH and by the Earl M. Bane Charitable Trust.)

238.7 **AN IN VIVO ASSAY OF INTRACELLULAR pH AND CALCIUM/CALMODULIN MODULATION OF cAMP PHOSPHODIESTERASE ACTIVITY.** D.J.Green and R.Gillette. Department of Physiology, Neural and Behavioral Biology Program, University of Illinois, Urbana, IL 61801

In vivo, the ventral white cells (VWCs) of Pleurobranchaea display intense minutes-long bursts of action potentials which are critical to the functional role of the VWCs in the consumatory phase of feeding (see Gillette and Green, this volume). Prolonged, endogenous bursting can be mimicked in vitro by intracellular cAMP injection.

Previous results from our lab have demonstrated that calcium and intracellular pH (pHi) modulate the excitability of the VWCs. We sought to examine the sensitivity of the major enzymes underlying the cAMP response - phosphodiesterase (PdE), protein kinase (PK), and phosphatase (PTase) - to the proton and calcium fluctuations that normally occur during bursting. Equivalent injections of cAMP induce an inward sodium current that describes a waveform with a characteristic and constant amplitude and timecourse. We found that selective chemical inhibition of each enzyme induces a characteristic change in the waveform. Notably, PdE inhibition with methylxanthines (IBMX) greatly increases the duration and peak amplitude of the current. PK inhibition via tolbutamide decreases the peak amplitude, with no affect on the duration. Oxidizes glutathione inhibition of PTase increases the peak amplitude, but, like PK and unlike PdE inhibition, does not affect the duration.

Using this in vitro assay of enzyme activities based on the shape of the current waveform, we found that slight intracellular alkalinization or acidification increased the peak amplitude and duration of the cAMP current response, which is consistent with a decrease in the PdE activity. Calmodulin inhibitors such as trifluoperizine (TFP) and W-7 mimicked the affects of IBMX and pHi changes. Depolarization-induced attenuation of the cAMP current was proportional to the extracellular concentration of calcium, and is consistent with an increase in PdE activity.

These results indicate that 1) certain cAMP-related enzymes can be monitored qualitatively in vivo and 2) PdE is the major site of action of pHi and calcium/calmodulin, and that PdE regulates the duration, termination, and interburst interval of cAMP-stimulated burst episodes in the VWCs. Supported by NSF BNS 8308551 to R.G.

238.8 THE EFFECTS OF AN ALKYLATING DERIVATIVE OF OXOTREMORINE (BM 123) ON MUSCARINIC RECEPTORS AND ADENYLATE CYCLASE ACTIVITY IN THE RABBIT MYOCARDIUM. F.J. Ehlert* (SPON: R.D. Crosland). Dept. Pharmacol. UCLA School of Med., Los Angeles, CA 90024.

The effects of a 2-chloroethylamine derivative of oxotremorine, N-[4-(2-chloroethylmethylamino)-2-butynyl]-2-pyrrolidone (BM 123), on heart rate, muscarinic receptor binding properties, and adenylate cyclase activity were investigated in the rabbit myocardium. Isolated hearts were perfused for 30 min with Ringer-Locke solution containing BM 123 (20 μM) and then perfused an additional 90 min without BM 123. Control hearts were perfused in the same manner except for exposure to BM 123. Following perfusion, the intact hearts were homogenized and assayed for muscarinic receptors and adenylate cyclase activity. Treatment with BM 123 caused an initial asystole followed by bradycardia during the 30 min perfusion. Heart rate recovered to control rate during the 90 min wash period. BM 123 treatment caused 75% alkylation of muscarinic receptors as measured by the reduction in the binding capacity of the specific muscarinic antagonist [$^3$H]N-methylscopolamine ([$^3$H]NMS). In control myocardial homogenates, $MgCl_2$ caused a concentration dependent stimulation of adenylate cyclase activity with the $ED_{50}$ for this effect being 2 mM. The $ED_{50}$ value of $MgCl_2$ increased 5 fold in the presence of the muscarinic agonist oxotremorine-M (100 μM). There was no significant difference in the potency with which $MgCl_2$ stimulated adenylate cyclase activity in control hearts and hearts which had been perfused with BM 123 and washed extensively. In control hearts, oxotremorine-M caused a maximal inhibition of adenylate cyclase activity of 46% with the $K_i$ for this effect being 0.079 μM. In hearts which had been perfused with BM 123, the maximum inhibitory effect of oxotremorine-M on adenylate cyclase activity was only 15% with the $K_i$ value being 0.56 μM. The irreversible effects of BM 123 on muscarinic receptor binding capacity and adenylate cyclase activity described above were not observed in hearts which had been perfused with BM 123 and atropine (1 μM) or with the reversible muscarinic agonist oxotremorine-M (20 μM/30 min). The results described here are consistent with the postulate that alkylation of muscarinic receptors with BM 123 has little effect on adenylate cyclase activity by itself but causes a functional blockade of muscarinic inhibition of adenylate cyclase activity.

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238.9 MUSCARINE STIMULATES INOSITOL-PHOSPHOLIPID METABOLISM AND INHIBITS ADENYLATE CYCLASE ACTIVITY IN PC12 CELLS. J. Horwitz*, M. Wang*, and R.L. Perlman. (SPON: R. Greenberg). Department of Physiology and Biophysics, University of Illinois College of Medicine, Chicago, IL 60680.

PC12 cells are clonal line of pheochromocytoma cells that have been widely used as a model to investigate the properties of adrenal chromaffin cells and sympathetic neurons. We and others have shown that muscarine increases the accumulation of $^3$H-inositol phosphates in PC12 cells in which the inositol containing phospholipids had been labeled by preincubation with $^3$H-inositol. Muscarine also increases the accumulation of $^3$H-monoacylglycerol and $^3$H-diacylglycerol in cells that had been preincubated with $^3$H-arachidonic acid. These data suggest that muscarinic stimulation increases the activity of phospholipase C in PC12 cells.

More recently, we have investigated the effect of muscarinic stimulation on adenylate cyclase in these cells. PC12 cells contain an adenosine-sensitive adenylate cyclase. Incubation of the cells with adenosine analogs, such as 2-chloroadenosine and phenylisopropyladenosine, causes a large increase in the accumulation of cyclic AMP. Muscarine inhibits phenylisopropyladenosine-induced accumulation of cyclic AMP by up to 70%. This effect of muscarine is presumably due to the inhibition of adenylate cyclase activity, since muscarine inhibits cyclic AMP accumulation in the presence of Ro-1724, a non-xanthine cyclic nucleotide phosphodiesterase inhibitor. The action of muscarine is blocked by atropine, and so is apparently due to the activation of muscarinic receptors in the cells. The EC50 for the inhibition of adenylate cyclase is between 1 and 10 μM. Thus, muscarinic stimulation increases phospholipase C activity and inhibits adenylate cyclase activity in PC12 cells. The relationship between these two effects of muscarinic agonists remains to be clarified. (Supported by NIH grants HL29025 to R.L.P. and HL06701 to J.H.)

238.10 ADENYLATE CYCLASE AND GTP-BINDING PROTEIN MEDIATE SENSORY TRANSDUCTION IN OLFACTORY NEURONS. Doron Lancet, Judith Heldman* and Umberto Pace*. Dept. of Membrane Research, The Weizmann Institute of Science, Rehovot, Israel.

The mechanism of olfactory transduction has been a subject of much controversy and speculation. Our recent studies provide strong evidence that odorants activate adenylate cyclase in the dendritic membranes of olfactory sensory neurons. The putative olfactory receptor molecules appear to be coupled to the transductory enzyme via a GTP-binding protein (G-protein). Our experiments are conducted on a preparation of isolated olfactory cilia (dendritic extensions of the sensory neurons), which contain the sensory molecular apparatus, similar to isolated retinal rod outer segments. The chemosensory cilia from amphibian and mammalian species are found to contain extremely high specific adenylate cyclase activity, 10-30 times higher than brain membranes. Odorants, either individually or in mixture, enhance cyclic AMP production by 120-250%, and they do so in a ligand and tissue specific way. This, together with previously published in-vivo electrophysiological evidence for cyclic nucleotide mediation, strongly support the role of adenylate cyclase in olfactory transduction. Odorant stimulation of ciliary adenylate cyclase occurs only in the presence of GTP, suggesting the involvement of a signal-coupling GTP-binding protein (G-protein). The olfactory G-protein can be identified by ADP-ribosylation with bacterial toxins, and appears to be similar in molecular weight and toxin specificity to the hormonal stimulatory G-protein ($G_s$). Previously published observations that pseudo-hypoparathyroidism patients, who are $G_s$-deficient (Farfel et al, PNAS 78, 3098 (1981)) have severely impaired olfactory thresholds (Henkin, J.Clin.Endocrynol.28,624 (1968)) further corroborate the role of $G_s$ in olfaction. Our results constitute the first direct identification of a molecular component involved in odorant reception, and suggest a similarity between olfaction, photoreception, hormone and neurotransmitter reception. Odorant sensitive adenylate cyclase and olfactory G-protein provide powerful tools for future identification and isolation of the elusive odorant receptor proteins (Chen et al., this volume). An open question is how cyclic AMP affects the odorant-controlled depolarizing ion channels of olfactory sensory neurons. Two important possibilities are cyclic AMP-dependent phosphorylation, and direct cyclic nucleotide gating. We find that olfactory cilia contain cyclic AMP dependent protein kinase and phosphorylated polypeptides, but at present there is no direct evidence for their role in olfaction. Future biochemical and single ion channel recording experiments should help resolve these questions.

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238.11 GUANINE NUCLEOTIDE EXCHANGE BETWEEN GTP-BINDING PROTEINS RESPONSIBLE FOR THE ACTIVATION OR INHIBITION OF NEURONAL ADENYLATE CYCLASE. M.M. Rasenick and M.M. Marcus*, Dept. of Physiology and Biophysics, U. of Illinois Coll. of Med. Chicago, IL 60680

Synaptic membrane adenylate cyclase (AC) can be activated or inhibited by various neurotransmitters, receptors for which exert their effects on AC through GTP binding proteins. A 42KDa GTP-binding protein (GNs) has been shown to stimulate and a 40KDa protein (GNβ) to inhibit, AC. A third 35KDa protein (NGβ) is associated with GNs and GNi and may regulate AC activity by interacting with these proteins, yet this process is not yet understood.

Hydrolysis-resistant GTP analogs promote activation of AC which persists after buffer washing of the membranes. These same analogs can promote stable inhibition of AC in membranes prepared from brain but not in those from non-neural tissues. We have employed the hydrolysis-resistant GTP photoaffinity analog 5'azdoanilido GTP to study the stable activation and stable inhibition of synaptic membrane AC as well as the interaction among GNs, GNβ and GNB during these processes. When synaptic membranes are incubated with AAGTP ($10^{-7}$ to $10^{-4}$ M) for 3 min at 23°C 1 mM $Mg^{++}$, inhibition of AC, which is stable to washing, results, as long as the membranes are assayed under conditions (23°C, 5 mM $Mg^{++}$) which favor continued expression of AC inhibition. If the membranes are exposed to increasing concentrations of the stable GTP analog, GppNHp under conditions which favor AC stimulation (30°C, 5 mM $Mg^{++}$), the stable AC inhibition is overridden and stimulation of the enzyme occurs. When $^{32}P$ AAGTP is incubated with membranes under the above (inhibitory) conditions and SDS PAGE and autoradiography are performed in the place of the AC assay, stable binding at the 40KDa GNi protein (which also serves as a substrate for pertussis-toxin catalyzed ADP ribosylation) occurs. Under the above conditions where the stable AC inhibition yields to stimulation of the enzyme the AAGTP bound to GNi (before UV photolysis) is shifted to the 42KDa GNs. Substitution of NaF for GppNHp results in a similar shift from AC inhibition to AC activation as well as a similar shift in AAGTP labelling from GNi to GNs. The shift in bound AAGTP is rapid (it occurs within 2 min) and it is stable for 20-30 min. The magnitude of the GNi-GNs shift corresponds to the predominant expression of enzyme activity (inhibition vs. stimulation) but the total $^{32}P$ DPM of AAGTP bound in GNs+GNi proteins reamins constant. Furthermore as the AA-GTP label bound to GNi increasingly switches to GNs, an increasing amount of AAGTP is associated with the GNβ protein. Although no binding site for GTP has been proposed for this protein, the proximity of GNβ to the GNs and GNi proteins may permit covalent binding of the photoaffinity compound. An increased association of the GNβ protein with the AC complex during the apparent exchange of AAGTP between GNi and GNs may serve as the mechanism for this exchange process, and as a biological switch for the activation and inhibition of neuronal AC.

238.12 FUNCTIONAL HOMOLOGY AND IMMUNOLOGICAL CROSS-REACTIVITY BETWEEN ROD OUTER SEGMENT AND SYNAPTIC MEMBRANE ADENYLATE CYCLASE GTP-BINDING PROTEINS. C.A.Moore*, H.E.Hamm and M.M.Rasenick (SPON: J.Mitrius) Dept. of Physiol. and Biophys. U. of Ill. Coll. of Med., Chicago, IL 60680.

Many recent studies indicate that there is a family of GTP-binding proteins with similar structure and function. Two such GTP binding proteins are found in the photoreceptor, where light-activation of cGMP phosphodiesterase (PDE) is mediated by a 39kDa protein (G), and in synaptic membranes where there is a hormone-activated (or hormone-inhibited) adenylate cyclase (AC) whose activity is mediated by a 42kDa ($GN_s$) or a 40kDa ($GN_i$) protein. There appears to be a structural homology (1), and functional interchangeability (2) between G and $GN_s$ and $GN_i$, and all three proteins interact with an apparently common 35kDa β subunit. Recently, a series of monoclonal antibodies has been generated against $G_\alpha$;one of these (4A) blocks light-activated GTP binding to G and PDE activation by the G-GTP complex (3).

In this study, we demonstrated that a mixture of several monoclonal antibodies to photoreceptor $G_\alpha$ specifically cross-react with $GN_s$ from rat synaptic membranes by binding to that protein on nitrocellulose blots. This cross-reactivity is more easily detectable for the subset of $GN_s$ which is solubilized by treatment of membranes with microtubule disrupting drugs (4), and is thus enriched in this fraction. The antibodies appear to cross-react with the β as well as the α subunit, suggesting some level of homology between the two subunits. (However, in the photoreceptor, the antibodies bind to the α subunit several orders of magnitude better than to the β subunit).

Functional studies also suggest a homology between photoreceptor and brain G-proteins. Incubation of 4A with synaptic membranes showed an antibody dose-dependent inhibition of AC, both basal and AC stimulated by GppNHp, $F^-$, forskolin, and $Mn^{++}$. The magnitude of the inhibition was approximately 1.5-2-fold in all cases. Similar results are obtained with pineal membranes, as previously reported (5), and with membranes prepared from C6 glioma cells. In all cases, the magnitude of the inhibition is about 2-fold, and in all cases, the largest inhibition is of the $Mn^{++}$-stimulated AC. Although $Mn^{++}$ is thought to act on the catalytic moiety of AC, in previous experiments reconstitution of $GN_s$ deficient AC by G augmented $Mn^{++}$ activation of AC. Thus, we are investigating the possibility that 4A may mitigate some interaction of $GN_s$ with the AC catalytic moiety. This apparent cross-reactivity between an antibody against G with $GN_s$ is interesting in light of the homology between the two systems, and may provide a valuable tool in studies of both the light- and neurotransmitter-activated systems.

1) J. Biol. Chem. 258:7059 2) PNAS 79:3408 3) J. Gen. Physiol. 84:265 4) Nature 294:560 5) Neurosci. Abst. 9:684

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## BIOLOGICAL RHYTHMS III

239.1 MEAL SELECTIONS BY HUMAN SUBJECTS IN TIME ISOLATION. C.P. Pollak* and M.L. Moline* (SPON: L. Schneider). Inst. Chronobiology, Dept. Psychiatry, The New York Hosp.-Cornell Med. Ctr., Westchester Div., White Plains, N.Y. 10605

While many circadian rhythms have been well characterized in human subjects studied in time isolation, the rhythms of meal selection are not completely understood (Aschoff, J., Wever, R., Wildgruber, C. and Wirz-Justice, A. Naturwissenschaften, 71:534, 1984). It is of interest, for example, to determine whether the timing of meals is related to the circadian pacemaker that is responsible for the core temperature rhythm, to a mechanism that controls the sleep-wake cycle, or to a third circadian clock. Human subjects have been studied in conditions of time isolation to begin to resolve this question.

Over forty subjects of both sexes between the ages of 16 and 81 have been studied. Each protocol begins with at least 4 days of entrainment (EN) to a subject's habitual sleep-wake cycle. During EN, four meals are scheduled per day: breakfast, lunch, dinner and snack. During free-running (FR), a subject chooses the timing of daily events, including all meals and sleep periods, and also the meal name and content. A detailed description of food requested and remaining as well as the time of request and the duration of the meal are recorded on a computer-based system. Each sleep period is recorded polygraphically. Analysis of meal naming and timing has begun in 9 of these subjects.

During FR, several changes in meal pattern occurred. The evening snack was dropped by most subjects and replaced by a later dinner. Lunch also occurred later in the circadian day, near the midpoint between sleep offset and sleep onset. Breakfast, by contrast, was requested shortly after awakening on each circadian day by most subjects with little or no change in the waking-to-breakfast interval between EN and FR. Cycle to cycle differences in the length of the circadian day appeared first in the time of sleep offset, followed by parallel changes in lunch time and sleep onset, and in dinner time and sleep onset as well in most subjects. The temporal organization of the waking day thus appeared to contract or expand homogeneously, depending on circadian day length. In those subjects who desynchronized during FR, inter-meal intervals increased in proportion to the increased day length. However, only three meals were selected, named by subjects "breakfast", "lunch" and "dinner" and taken in that order. The meal pattern therefore appeared to be synchronized with the sleep-wake cycle, even when its period exceeded 30 hours.

The results of these studies suggest that lunch and dinner selection may be influenced by timing mechanisms closely related to those that control the sleep-wake cycle. The decision to have breakfast may be more influenced by the awareness of just having slept than by timing mechanisms.

239.2 FOOD AVAILABILITY AS A POTENTIAL ZEITGEBER IN DASYURID MARSUPIALS. G.J. Coleman*, H.R. O'Reilly* and S.M. Armstrong*. Spon: (Dr. F. Guldner). Department of Psychology, La Trobe University, Bundoora, Vic., 3083., Australia.

Past research has shown that there is a circadian oscillator in laboratory rats which is entrained by restricted feeding (Boulos, Z., Rosenwasser, A.M. and Terman, M., Behav. Brain Res., 1:36-65, 1980). However, restricted feeding does not compete with the LD cycle in entraining wheel-running activity rhythms.

Given that Dasyurid marsupials are predominantly carnivorous, the episodic intake of food in the wild and the high nutritive content of that food suggests that food may be an important zeitgeber in these species. A variety of feeding schedules were used to determine whether such schedules would entrain circadian activity rhythms in competition with an LD cycle and also in constant dark (DD) conditions.

Six Kowari (Dasyuroides byrnei), desert dwelling nocturnal marsupials were fed at 1600 hr under a 12:12 LD cycle with lights on at 0600 hr (Stage 1). The meal was shifted to 0930 hr for 57 days (Stage 2), then DD was introduced for 117 days (Stage 3) with no change to the meal-time. Ad lib feeding in DD was then introduced for 58 days (Stage 4). While three Kowari showed activity anticipatory to the meal in Stage 2, the meal did not entrain any animals in stage 3 although there was some evidence of relative co-ordination.

In a second experiment, six Sminthopsis crassicaudata were maintained on an ad lib diet of pet food, presented with a much-preferred meal of mince meat mixture and exposed to a sequence of conditions similar to that in the previous experiment. In none of the conditions did any S. crassicaudata show anticipatory activity to the more palatable meal. There was some evidence of relative co-ordination in Stage 3 and in one animal, there was limited evidence for entrainment to the meal.

In the third experiment, 12 Sminthopsis macroura froggatti were presented with a daily meal at 0900 hr under a 12:12 LD cycle with lights on at 0600 hr for 37 days. Following this, all animals were exposed to periods of 12-18 days ad lib food interleaved with 3-day periods of deprivation, a technique used previously to demonstrate persistent meal-associated rhythms (Coleman, G.J., Harper, S., Clarke, J.D. and Armstrong, S.M., Physiol. Behav., 29:107-155, 1982). The meal-associated activity rhythms previously observed in rats were not seen, but the 3-day deprivation period produced large phase-shifts in the activity rhythms of several S. m. froggati.

It was concluded that meal-feeding does not compete with the LD cycle in entraining dasyurid marsupials, but that the frequent observation of relative co-ordination and phase shifts suggest a different and, perhaps, stronger role for food intake in biological rhythmicity than has been previously observed in rats.

239.3 EFFECTS OF PARTIAL SCN LESIONS ON BODY TEMPERATURE, DRINKING, AND ACTIVITY RHYTHMS IN RATS. Rebecca A. Prosser* and Evelyn Satinoff (SPON: J. Malpeli). Dept. of Psych., Univ. of Illinois, Champaign, IL 61820.

Complete suprachiasmatic nuclear (SCN) lesions which eliminate drinking and activity rhythms do not abolish the circadian temperature rhythm (CTR), but do decrease its amplitude and advance its acrophase in LD, and shorten the free-running period in DD (Prosser et al., THERMAL PHYSIOLOGY, 1984). Here we describe the effects of partial (25-90%) SCN lesions on these rhythms in 5 hooded rats for 8-10 months postoperatively. Body temperature (Tb) was measured every 10 minutes via implanted telemetry devices. The changes in the CTR after partial lesions were similar to but smaller than those seen after complete lesions: the amplitude decreased 0.6 ± .07°C (X ± SEM), the acrophase advanced 33.8 ± 10.9°, and the free-running period decreased from 24.27 ± .38 to 23.75 ± .52 hours. In one rat, the free-running period increased from 23.95 to 24.28 hours. There were significant decreases in nocturnal drinking (from 73 ± 3.1% to 56 ± 3.7%) and activity (from 67 ± 1.4% to 56 ± 2.6%). Drinking became arrhythmic in all 5 rats for varying amounts of time. In one rat it remained arrhythmic for the duration of the experiment (over 8 months). Four rats regained weak drinking rhythms by 2-10 weeks. Two of these rats regained stronger drinking rhythms by 10-20 weeks.

Activity became arrhythmic in 3 of the 5 rats for varying amounts of time. The two rats that remained rhythmic were those which regained the strongest drinking rhythms postsurgery. Two rats lost rhythmicity in activity for only 10 days. In one of these drinking remained aperiodic and in the other there was a weak drinking rhythm after 10 weeks. The 5th rat showed intermittent periods of rhythmic and arrhythmic activity. This rat (which had the increase in free-running period postlesion) regained a weak drinking rhythm after 10 weeks. Thus, in most rats, the drinking rhythm was more easily disrupted and remained arrhythmic longer than the activity rhythm. (Three other rats had drinking and Tb monitored after partial lesions. The drinking rhythm was permanently eliminated in one rat and temporarily eliminated for 10-25 days in the other 2 rats. Tb was rhythmic in all three rats.)

These results indicate that the drinking, activity, and Tb rhythms can be affected differentially by partial SCN lesions. Thus, the circadian pacemaker system of rats can be divided into several components, within as well as outside the SCN.

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239.4 DAMPED OSCILLATION OF THE LATERAL HYPOTHALAMIC MULTIPLE UNIT ACTIVITY INDUCED BY RESTRICTED FEEDING SCHEDULES IN RATS WITH SUPRACHIASMATIC NUCLEI LESIONS. S. Kurumiya* and H. Kawamura. Lab. of Physiol. Psychol., Dept. of Neuroscience, Mitsubishi-Kasei Inst. of Life Sci., Machida-shi, Tokyo 194, Japan.

In rats with suprachiasmatic nuclei (SCN) lesions, appearance of anticipatory wheel-running activity under restricted feeding schedules (RFS) and its persistence for several days during the subsequent food deprivation period has been reported (Stephan, F. K. et al., Behav. Neural Biol., 25: 545, 1979).

To investigate the neural mechanism for such a phenomenon, we studied multiple unit activity (MUA) of the hypothalamic feeding area.

In Wistar strain male albino rats with bilateral SCN lesions, two bipolar electrodes for MUA recording were implanted stereotaxically under pentobarbital anesthesia into the lateral hypothalamic area (LH) and ventromedial nucleus (VMH). Motor activity of the animal was measured using a capacitance change between a piece of open-ended wire attached on top of the head and a ground electrode. MUA and movements of the animal were counted electronically every 5 min and every 10 min respectively, and printed out continuously. After recovery from surgery, the rat was placed in a sound-attenuated, temperature controlled room under constant darkness. Water was available at all time.

During 5 days control recording period, when food was always available, no daily rhythm was found in rats with complete SCN lesions in both brain MUA and motor activity. However, during RFS when food was available only for 2h (from 14:00 to 16:00), MUA of the LH indicated gradual increase prior to the beginning of the feeding time and reached to a peak during feeding and decreased thereafter. Such changes indicated a potentiation toward the end of the RFS. It persisted for several days even during food deprivation period after termination of RFS and quickly disappeared. Changes of MUA in VMH was not as significant as in LH.

These findings may suggest existence of, not self-sustained but quickly damping oscillator in the lateral hypothalamic feeding center. Such oscillation was induced only when the RFS was applied with definitely regular periods close to 24h, and was quite different from the self-sustained oscillation induced by SCN.

239.5 DISRUPTION OF CIRCADIAN ACTIVITY RHYTHMS IN THE RAT BY MIDBRAIN RAPHE LESIONS. J. Levine†, A.M. Rosenwasser, J.A. Yanovski†, and N.T. Adler. Dep't of Psychology, University of Pennsylvania, Philadelphia PA 19104.

The midbrain raphe nuclei provide a robust serotonergic input to the suprachiasmatic nuclei (SCN) of the hypothalamus, a putative circadian "master oscillator." This projection may comprise part of a neural network linking the retina, SCN, ventral lateral geniculate, and raphe nuclei. These observations suggest that the raphe may play a role in the coordination of the circadian timing system. An earlier study (Block and Zucker, 1976) showed that raphe lesions could reduce the clarity of circadian activity rhythms in the rat. However, all animals displayed detectable free-running rhythms. Therefore, in the present study we re-examined the effects of raphe lesions on circadian activity rhythms in the rat.

Electrolytic lesions were stereotaxically aimed at the median raphe nucleus. Although post-operative mortality was high, we were eventually able to produce a group of ten such animals. These animals were subsequently maintained in running wheel cages under light-dark 12:12, constant light, and constant dark conditions. Each lighting condition was in effect for at least one month. Under the light-dark cycle all animals showed primarily nocturnal activity. However, under constant conditions five animals showed severely disrupted activity rhythms, while the other five showed more normal free-running rhythmicity.

Histological analysis revealed clear differences between the lesions which disrupted free-running rhythms and those which did not. The effective lesions were generally larger and more dorsocaudally placed, relative to the ineffective lesions. As a group, the ineffective lesions caused extensive damage to the interpeduncular nuclei and relatively less damage to the median raphe. In addition, the dorsal-most extent of the median raphe was spared in all these lesions. In contrast, the effective lesions caused quite extensive damage to the median raphe, and also involved the ventral aspect of the dorsal raphe. These lesions also usually damaged the ventral tegmental nuclei. Overall, it appeared that the area between the median and dorsal raphe nuclei might be critical for the display of free-running circadian activity rhythms.

These results support the hypothesis that the raphe nuclei, and presumably their projections to the SCN, play a major role in the control of circadian activity rhythms. While the exact nature of this role cannot yet be specified, it seems plausible that the raphe nuclei contribute to the maintainance of oscillator coupling in a multi-oscillator neural circadian timing system.

239.6 INTERGENICULATE LEAFLET LESIONS ALTER PHOTIC RESPONSES OF HAMSTER CIRCADIAN RHYTHMS. M.E. Harrington and B. Rusak. Dept. of Psychology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1.

The suprachiasmatic nuclei (SCN) function as a dominant oscillator in the mammalian circadian system (Rusak, B. & Zucker, I., Physiol.Rev. 59:449-526, 1977). In hamsters, the SCN receive putative photic input both directly from the retina and indirectly from the intergeniculate leaflet (IGL) in the thalamus (Pickard, G.E., J. Comp.Neurol. 211:65-83, 1982). We have investigated the effects of destruction of the IGL on photic responsiveness of the circadian system of golden hamsters.

Eleven intact hamsters were housed in constant darkness; phase shifts in running wheel activity rhythms were assessed following 15 min light pulses (40 $\mu W/cm^2$). Pulses were administered at four phases: circadian time (CT) 12 (defined as the beginning of activity), CT14, CT18 and CT20. Eight animals subsequently underwent ablation of the IGL while 3 served as intact controls. Responses to light pulses at the same CT's were assessed and compared to pre-lesion shifts. Six hamsters with complete IGL lesions showed decreases in phase advances caused by light pulses at CT18 and CT20. Advances to pulses at CT20 were reduced by 89 ±35 min in ablated animals and .5 ±22 min in controls. Phase delays elicited by light at CT12 and CT14 were not altered.

In a second study, 20 hamsters were housed in constant light (LL). Ten underwent ablation of the IGL while 10 were intact controls. Six hr dark pulses were given at various CT's and phase shifts of running-wheel activity rhythms were assessed. Eight animals with complete IGL lesions showed smaller advances than controls to dark pulses centered on CT9 and CT17. During 110 days in LL, 7 out of 10 intact animals showed typical "splitting" of activity into two components, while only 1 of the 8 ablated animals displayed dissociated activity components. Possibly related to this difference in propensity to show splitting was the finding that ablated animals had significantly shorter free-running periods in the first 35 days of LL, before any dark pulses were given, than did the intact animals (24.07± .08 vs. 24.26±.18; t-test, $p<.02$).

These results demonstrate that destruction of the IGL in the hamster alters phase-shifting in response to periods of light or dark, and they indicate a role for the IGL in mediating several photic effects on the circadian system.

Supported by MRC and NSERC of Canada and Dalhousie RDFS.

239.7 **RESETTING THE CIRCADIAN CLOCK IN THE SUPRACHIASMATIC BRAIN SLICE.** M.U.Gillette. Department of Physiology and Biophysics and the Neural and Behavioral Biology Program, University of Illinois, Urbana, IL 61801.

Neurons of the suprachiasmatic nuclei (SCN) of the hypothalamus comprise a primary oscillator which organizes circadian rhythms in mammals. The ability to keep 24 hour time, expressed most directly as an oscillation in neuronal firing rate, is an endogenous property of these neurons and survives isolation using the hypothalamic brain slice technique. However, I have found that the oscillator is sensitive to the phase of circadian time at which the brain slice is prepared: it is reset during preparation at sensitive times and the phase change is maintained up to 32 hours in vitro.

I systematically examined the relationship of circadian time of suprachiasmatic isolation and the phase of the next oscillation in firing rate of SCN neurons in vitro. Suprachiasmatic brain slices were prepared from a total of seventeen Long-Evans rats at a range of times in their 12L:12D circadian cycles. In each experiment, single unit activity of 50-96 neurons in paired SCN, isolated in a single 400 μm brain slice, was sampled over 12-28 hours.

For SCN in hypothalamic slices prepared during the donor's day, single unit activity continued unperturbed in phase, with a maximum and period similar to those observed both in animals implanted with chronic multiunit electrodes and in previous studies on isolated SCN. Isolation of the SCN during the dark portion of the circadian cycle, however, produced marked changes in the phase of the next oscillation. SCN isolation during the first 4 hours of the donor's night produced a delay of up to 4 hours in the peak of the next oscillation in electrical activity. At later times in the dark period, brain slice preparation advanced the phase up to 4.5 hours. The sign and shape of the phase-response relationship for resetting neural activity rhythms in the isolated oscillator is very similar to that for light- or electrode-stimulated resetting of circadian behavioral rhythms in intact animals.

The faithfulness of the phase-shift data from the isolated SCN to that for intact animals indicates that the resetting mechanism is endogenous to the SCN, and that when stimulated, it proceeds normally in vitro. This establishes the usefulness of the suprachiasmatic brain slice for probing the mechanism of the circadian oscillator. Supported by NSF BNS 83-08551 and PHS-BRSG-RR7030.

239.8 THE SUPRACHIASMATIC NUCLEI CONTAIN A TETRODOTOXIN-RESISTANT CIRCADIAN PACEMAKER. W.J. Schwartz, R.A. Gross*, M.T. Morton*. Neurology Svc., Mass. Gen. Hosp. & Harvard Med. Sch., Boston, MA 02114.

Two complementary measures of functional activity, in vivo glucose utilization & multiple unit activity, have helped to establish that the suprachiasmatic nuclei (SCN) contain a working circadian pacemaker. However, energy metabolism in the SCN is already oscillating with a circadian rhythm prenatally, whereas SCN firing rates first appear rhythmic weeks later postnatally. Therefore, SCN action potentials do not appear to be part of the internal timekeeping mechanism of the pacemaker; rather, electrical impulses might function to couple the pacemaker to its input pathways for entrainment and its output pathways for overt rhythm expression. To test this hypothesis, we chronically infused tetrodotoxin (TTX) for 14 d into the SCN of unrestrained rats. TTX blocks voltage-dependent $Na^+$ channels and SCN action potentials recorded from slices in vitro (Neurosci. Abstr. 10:294, 1984).

In the first experiment, adult male rats were entrained to a light-dark (LD) cycle for 2 wks before they were blinded. Free-running drinking rhythmicity was recorded for 10 d, after which cannula assemblies were stereotaxically inserted into the SCN and connected to subcutaneously-implanted mini-osmotic pumps filled with TTX in normal saline or artificial CSF. Control rats received infusions of vehicle alone. Free-running rhythms were recorded during the infusions and for 1 month after pumps were exhausted.

When 1 μM TTX was infused into the SCN, drinking arrhythmicity occurred. Control rats or those with cannulae outside the SCN continued to drink rhythmically. When TTX infusions ended, drinking rhythms resumed with free-running circadian periods ($\tau$) unaltered from those expressed pre-infusion. Phase of the restored rhythms was that predicted by extrapolation of the original (pre-infusion) $\tau$. That is, TTX infusions caused no phase shifts.

In the second experiment, rats were entrained and cannulae inserted as above. During the infusions the animals were exposed to an LD cycle progressively delayed until a completely reversed cycle was attained. When infusions ended, rats were placed in constant darkness and drinking rhythms recorded for 1 month.

In control rats, overt drinking rhythms were phase-shifted by the new LD cycle. On the other hand, TTX prevented this phase-resetting action of light, and no phase shifts occurred. That is, the phase of the restored (post-infusion) rhythms was that expected had the rhythms continued to free-run despite application of the reversed LD cycle.

Therefore, TTX blocked function of both the input & output pathways without disturbing the pacemaker's oscillatory mechanism. $Na^+$-dependent action potentials in the SCN appear to be necessary for the entrainment & expression of overt circadian rhythms, but they are not required for the pacemaker to keep accurate time.

239.9 CIRCADIAN CLOCK IN CELL CULTURE: OSCILLATION OF MELATONIN RELEASE FROM PERIFUSED CHICK PINEAL CELLS. L. M. Robertson* and J. S. Takahashi. Department of Neurobiology and Physiology, Northwestern University, Evanston, IL 60201.

The avian pineal gland contains circadian oscillators that regulate rhythmic release of melatonin. We have developed a dispersed pineal cell culture system in order to begin a cellular analysis of this vertebrate circadian oscillator. Ten chick pineal glands were dispersed with collagenase yielding approximately 2 x $10^5$ cells per gland. The dispersed cells were combined with cytodex-3 beads and cultured in Medium-199 for one week in LD 12:12. During this time the cells attached to the beads and sent out processes. Aliquots were then loaded into 3-5 replicate cell chambers and perifused 0.5 ml/hr with Medium-199. Two-hour fractions were assayed for melatonin content using radioimmunoassay. Initially, the cells were exposed to LD 12:12 for one day followed by constant darkness for four days. Pineal cells exhibited an increase in melatonin release during the first 12 hours of darkness followed by a decrease during 12 hours of light. The melatonin oscillation persisted for four cycles in constant darkness with a period close to 24 hours. The endogenous oscillation exhibited a gradual decrease in amplitude and increase in peak width. In contrast, cells maintained on LD 12:12 for five days expressed a high amplitude oscillation with no dampening of the rhythm. Because the melatonin oscillation from parallel cell chambers did not dampen in LD 12:12, the dampening observed in constant darkness is not due to unfavorable culture conditions but appears to result from a gradual desynchronization of multiple oscillators within the culture. Exposure of pineal cells to three days of constant light caused a strong dampening of the melatonin rhythm. Surprisingly, the overall level of melatonin released was not lower than that found in constant darkness. The apparent lack of light inhibition in constant light could be reversed by return of the cultures to LD 12:12. The melatonin oscillation was present in cells maintained for over one month in culture. This suggests that the components of the oscillating system remain intact in vitro for long periods of time.

In summary, dispersed pineal cells exhibit an endogenous oscillation of melatonin release. The oscillation among replicate cultures was very reproducible and consistent. The rhythmicity persists for at least four cycles with a period of close to 24 hours and is entrainable to a light-dark cycle. The dispersed cell perifusion system should provide an excellent model for a cellular analysis of mechanism. (Supported by Alfred P. Sloan fellowship BR-2366 and NIH grant MH 39592)

240.1 PURIFICATION AND CHARACTERIZATION OF ACETYLCHOLINESTERASE FROM DROSOPHILA MELANOGASTER. A. Gnagey*[1], T. Rosenberry*[2], and M. Forte[1]. [1]Dept. of Biology and [2]Dept. of Pharmacology, School of Medicine, Case Western Reserve University, Cleveland, OH 44106.

The Ace locus in Drosophila is important for neuronal function and development. Mutations and chromosomal rearrangements in this locus affect the production of acetylcholinesterase (AChE). We have purified AChE from wild-type Drosophila and determined its molecular forms in order to compare the enzyme produced by various Ace mutants at the developmental and molecular level. Purification of AChE was accomplished by one-step affinity chromatography according to the method of Rosenberry and Scoggin (J. Biol. Chem. 259:5643, 1984). Using this procedure, we have purified the enzyme over 10,000 fold with a 75 to 80% recovery of the initial enzymatic activity. From 50 gm of fly heads, we can prepare approximately 1 mg. of purified enzyme. In the reduced form, this material shows a single major band at 52Kd; the non-reduced form is 110Kd. Since these values are significantly lower than those observed for AChE from vertebrate tissues, the extent of proteolytic cleavage has been assessed by covalently modifying AChE in freshly prepared homogenates with the specific label, $^3$H-diisopropylphosphofluoridate. Following SDS-PAGE and fluorography, molecular weights for the reduced form are similar to those observed for the purified material (52K Daltons).

In many animal species, AChE exists in several forms which have distinct sedimentation coefficients on sucrose gradients. In Drosophila, two forms are present; a major form which sediments at approximately 6.6S in the presence of TX-100 and a minor form at 4.0S, presumably indicating the dimer and monomer respectively.

Monoclonal antibodies to Drosophila AChE have been produced following in vivo immunization of mice with the purified enzyme. To date at least 17 different cell lines producing antibody against AChE have been identified. These monoclonals are currently being characterized and should be useful in examining the developmental expression of AChE in both wild-type and Ace mutants.

Supported by grants from NSF and NIH.

240.2 MOLECULAR FORMS OF ACETYLCHOLINESTERASE AND BUTYRYLCHOLINESTERASE IN THE HUMAN CENTRAL NERVOUS SYSTEM. J.R. Atack and E.K. Perry. (Spon. P.B. Guthrie). Lab. of Neurosciences, NIA, Bldg 10, NIH, Bethesda, MD 20205 and Dept. of Neuropathology, Newcastle General Hospital, Newcastle upon Tyne, England

In the central nervous system (CNS), whilst acetylcholinesterase (AChE; EC 3.1.1.7) is presumed to hydrolyse acetylcholine (ACh) at cholinergic synapses, the physiological function of butyrylcholinesterase (BChE; EC 3.1.1.8) remains unknown. Both enzymes exist as homologous series of six molecular forms that are polymers of a single, catalytically-active subunit. These molecular forms are designated G1, G2, G4 (globular forms) and A4, A8 and A12 (asymmetric forms) according to the absence or presence of a collagen-like "tail" and the number of subunits present. The distribution of these different molecular forms varies from tissue to tissue in a manner that is thought to somehow reflect the physiological function of that particular tissue (Massoulie and Bon, Ann. Rev. Neurosci., 5:57, 1982). Thus, for example, different distributions of AChE have been reported to occur in fast and slow rat muscle (Groswald and Dettbarn, Neurochem. Res., 8:983, 1983). In light of these observations, the present study was undertaken to determine whether or not specific distributions of both AChE and BChE molecular forms occur in relation to distinct anatomical and neurochemical features of the human CNS. The regions selected for these studies were the cerebrospinal fluid (CSF) and, in descending order of specific AChE activity, the postmortem; caudate nucleus, nucleus basalis of Meynert, substantia nigra, cerebellum, spinal cord, hippocampus, temporal cortex, fornix and parietal cortex - range 28 to 0.5umol/min/g wet weight. In contrast, the levels of BChE activity varied much less from region to region - range; 0.72 to 0.25umol/min/g wet weight in the cerebellum and parietal cortex respectively.

In all regions studied, including the CSF, the majority of the AChE activity was present as the G4 form with the G1 form constituting most, if not all (depending on the region), of the remaining activity. The ratio of G4:G1 AChE varied from 21 in the caudate nucleus to 1.7 in the temporal cortex. Similar to AChE, BChE was present as both the G4 and G1 forms. However, the ratio of G4:G1 BChE varied much less from region to region - range, 3.3 to 0.9 in the substantia nigra and temporal cortex respectively.

The observations that the G4 form of AChE was the major form associated with both cholinergic and non-cholinergic structures suggests that this particular form is functionally important not only in the hydrolysis of ACh at the cholinergic synapse but in the other physiological functions of AChE in the CNS (Greenfield, TINS, 7:364, 1984). Since there was a relatively even distribution of BChE and its molecular forms throughout the widely differing regions of the CNS examined, we were unable to deduce any physiological function for this enzyme.

240.3 EFFECT OF VARIOUS SYNTHESIS INHIBITORS ON THE DYNAMICS OF DOPAMINE AND ITS METABOLITES IN THE RAT AND MOUSE CORPUS STRIATUM. P.S. McQuade, J.W. Richard* and M. Thakur*. Douglas Hospital Research Centre and Dept. of Psychiatry, McGill University, Verdun, Quebec H4H 1R3 Canada.

The relationship between the synthesis of a neurotransmitter and its release has been investigated using a great many paradigms. In our model, the simultaneous measurement of dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and dopamine (DA) concentrations reflect the total synthesis of the neurotransmitter while the concentrations of 3-methoxytyramine (3-MT) are an index of any changes in the functional release of DA. Since the dynamics of dopamine metabolism and 3-MT changes, in particular, are very different in the rat and the mouse, a number of synthesis inhibitors were compared in these two species. The animals were microwaved after treatment, the striata were removed and DA, HVA, DOPAC and 3-MT were measured simultaneously by negative chemical ion gas chromatography-mass spectrometry. α-methyl para-tyrosine (250 mg/kg; i.p.), an inhibitor of tyrosine hydroxylase, produced a rapid decline in DA concentrations twenty minutes after injection in both species while decrease in 3-MT concentrations only commenced between 1 and 2 hrs. DOPAC concentrations declined simultaneously with DA concentrations. Interestingly 3-MT concentrations were maximally reduced in the rat by 2 hrs while in the mouse the decline continued at 4 hrs. NSD-1015, (100 mg/kg; i.p.), an inhibitor of L-DOPA decarboxylase, reduced DA concentrations only in the rat striatum at 1 and 2 hrs. This inhibitor, however, reduced DOPAC and HVA concentrations in both species while increasing 3-MT concentrations. NSD-1015 may thus interfere with either the oxidation of DA or its reuptake from the synaptic cleft. Since the dynamics of 3-MT are so vital to our model, tropolone, (50 mg/kg; i.p.), a COMT inhibitor, was employed over a very short time course. 3-MT is more rapidly removed in the rat than in the mouse. HVA concentrations decline more rapidly in the mouse than in the rat while DOPAC accumulates more quickly in the rat. This suggests that in the rat a greater proportion of DA is metabolized via the DOPAC pathway. These studies demonstrate that the concentrations of 3-MT are not immediately sensitive to the inhibition of DA synthesis thus suggesting that these neurons possess considerable reserves of releasable DA following DA synthesis inhibition. In contrast DOPAC concentrations are very sensitive to DA synthesis inhibition even in the mouse where the proportion of DA processed via the DOPAC pathway appears to be smaller than in the rat. (Supported by the Medical Research Council of Canada and the Douglas Hospital Research Centre).

240.4 WHOLE BODY TURNOVER OF CATECHOLAMINE IN CHRONIC SCHIZOPHRENIA AND DEPRESSION: EVIDENCE OF LOW DOPAMINE TURNOVER IN CHRONIC SCHIZOPHRENIA. F. Karoum, C. Karson*, W. Lawson*, A. Roy* and R.J. Wyatt. Neuropsychiatry Branch, NIMH, Intramural Research Program, Saint Elizabeths Hospital, Washington, D.C. 20032

Whole body turnover of norepinephrine (NE) (sum NE) and dopamine (DA) (sum DA) were measured in a group of 43 chronic schizophrenic patients, 10 depressives with melancholia and 10 without melancholia. Age and sex matched controls were also studied. Whole body turnover of the amines were measured by adding up the molar excretion of the amines and their metabolites. The results obtained showed that in chronic schizophrenia sum DA but not sum NE is significantly lower than normal controls. As a result, the ratios of sum DA/sum NE were also significantly lower in the chronic schizophrenic patients as compared to their age matched controls. Treatment with haloperidol normalizes these ratios. In depressed patients with melancholia, sum NE but not sum DA was significantly high leading also to low ratios of sum DA/sum NE but this time the low ratios were due to elevated sum NE. No differences in sum DA, sum NE and their ratios were detected between the depressed patients without melancholia and their appropriate sex and age matched controls. The results of the present study suggest schizophrenia is associated with a hypoactive peripheral dopaminergic activities; a phenomenon that may also applied to brain dopaminergic systems. In contrast, while depression appears to be associated with normal peripheral dopaminergic systems, peripheral noradrenergic systems appear to be hyperactive. The possibility that a similar situation occurs centrally is suggestive from several reports on noradrenergic receptors down regulation by antidepressants. It is concluded that the assessment of whole body turnover of catecholamines offers a better index to changes in the amines disposition than would measurements of individual amines or their metabolites.

240.5 AN ALPHA TWO ADRENERGIC TONIC INHIBITION OF CATECHOLAMINE METABOLISM IN THE LOCUS COERULEUS OF BEHAVING RATS : EVIDENCES FROM IN VIVO VOLTAMMETRY. L. Quintin,F. Gonon*,M. Buda*, G. Hilaire*, C. Bardelay*, M.Ghignone* and J.F. Pujol*. INSERM U171, 69230 St Genis Laval and Department of Anesthesiology, Hôpital Cardiologique, 69394 Lyon, France.

One of the various regulations controlling the noradrenergic (NA)-locus coeruleus (LC) cell activity has been demonstrated to be alpha-2 adrenergic specific on the basis of electrophysiological data only in anesthetized rats (Svensson et al., Brain Res. 1975, 92 : 291-306, Marwaha et al., J. Pharm. Exp. Ther., 1982, 222 : 287-293). To assess the existence of this inhibition, under rigorously chronic conditions, recordings of LC catechol metabolic activity were performed with in vivo differential pulse voltammetry (Buda et al.,Brain Res.,1983, 273, 197-206 ; Gonon et al., 1983, 273, 207-216). A guiding cannula was implanted in the LC under chloral hydrate anesthesia. After 48h of recovery, in the laboratory, a newly treated and tested carbon fibre electrode was threaded through this cannula to monitor LC catechol oxidation current. Baseline values (100%) were taken as the mean of the absolute height of the 6 catechol peaks recorded during the last 10 min of a 60 min stabilization period. Results are expressed as variation of catechol peak height (% of baseline). Piperoxane 60 $mg.kg^{-1}$ i.p. and yohimbine 10 $mg.kg^{-1}$ i.p. induced a large increase (200% above baseline values) in catechol oxidation current. This increase was dose-dependent : experiment with graded doses of piperoxane i.p. (1 to 100 $mg.kg^{-1}$) allowed determination of a dose-response curve (log-probit method) with an ED50 = 29.7 $mg.kg^{-1}$ piperoxane i.p. These changes in catechol oxidation current noticed in behaving rats were confirmed by combined LC electrophysiological and electrochemical recordings in anesthetized rats (Hilaire et al., Soc. Neurosci. Abstr, 1981, 10 : I-679) and HPLC determinations on LC microdissections. In contrast guanfacine (1 $mg.kg^{-1}$) and clonidine (10 to 200 $ug.kg^{-1}$ i.p.) induced a dose-dependent decrease in LC catechol metabolic activity. Clonidine 50 $ug.kg^{-1}$ i.p. reversed the increase in catechol oxidation peak induced by piperoxane 30 $mg.kg^{-1}$. At variance to the effects induced by alpha-2 agonists and antagonists, prazosin, a highly selective alpha-1 antagonist, only evoked a small increase in catechol oxidation peak (10% above saline effect, p below 0.05). This weak effect is in agreement with the low density of alpha-1 binding sites in the LC (Young et al., PNAS, 1980, 77 : 1696-1700) and may be explained by the hypotension secondary to prazosin. The pharmacological modifications of LC catechol metabolic activity induced by alpha-2 selective drugs, in behaving rats, confirm the existence of an alpha-2 adrenergic specific inhibition on NA-LC cellular activity.

240.6 3-HYDROXYANTHRANILIC ACID OXYGENASE: ACTIVITY IN LESIONED RAT STRIATUM AND IN HUMAN BRAIN TISSUE. R. Schwarcz, R.J. White and W.O. Whetsell, Jr.[1] Maryland Psychiatric Research Center, P.O. Box 3235, Baltimore, MD 21228 and [1]Dept. of Pathology, Vanderbilt University Medical Center, Nashville, TN 37232.

3-Hydroxyanthranilic acid oxygenase (3HAO), the enzyme responsible for the biosynthesis of the endogenous excitotoxin quinolinic acid, has been identified in the rat brain (Abst. Soc. Neurosci. 10, 11.4, 1984). Following its initial characterization, we have now examined the effects of excitotoxic striatal lesions on 3HAO activity in an attempt to elucidate the cellular localization of the enzyme.

Ibotenic acid (IBO, 40 µg/2 µl) was injected unilaterally into the rat striatum to cause degeneration of local nerve cell bodies. Groups of animals were killed at various intervals following the IBO injection and their striata processed for the determination of 3HAO and, as a marker for the success of the lesion, glutamic acid decarboxylase (GAD) activity. The data (N=6 for each group) are expressed as a percentage ± S.E.M. of the contralateral, non-injected striatum (**p<0.01, paired t-test).

| Days after IBO | 3HAO | GAD |
|---|---|---|
| 2 | 136 ± 27 | 53 ± 13** |
| 7 | 439 ± 44** | 55 ± 18** |
| 21 | 316 ± 37** | 50 ± 10** |

Kinetic analyses revealed that the increased 3HAO activity was due to $v_{max}$ changes. This, as well as the time curve of the increase, suggests that the phenomenon constitutes a tissue reaction to excitotoxin-induced nerve cell loss. It is likely that the newly synthesized enzyme is contained in reactive glial cells.

3HAO activity could also be identified in human brain tissue obtained post mortem from subjects who had died without any recognized neurological or psychiatric disease. 3HAO was clearly detectable in those brains (N=9) and showed a 7-fold variation between the areas of highest and lowest activity. In the order of decreasing activity, the enzyme was measured in the medulla (absolute enzyme activity, determined at $v_{max}$:81±16 pmol quinolinic acid formed/hour/mg tissue), substantia nigra, hypothalamus, caudate, globus pallidus, thalamus, putamen, cerebellum, frontal cortex, hippocampus and parietal cortex. These data provide baseline values for future studies of 3HAO in the brains of victims of a spectrum of neurodegenerative disorders.

This work was supported by USPHS grants NS 16102 and 20509.

240.7 RADIOENZYMATIC DETERMINATION OF QUINOLINIC ACID. E. Okuno, A.C. Foster and R. Schwarcz. Maryland Psychiatric Research Center, P.O. Box 3235, Baltimore, MD 21228.

Recent experimental evidence has given rise to the idea that a pathological overabundance of the tryptophan metabolite quinolinic acid (QUIN) may be related to the etiology of neurodegenerative disorders in humans. (Life Sci. 35, 19, 1984). To date, the elucidation of such a possible role of QUIN has been severely limited by the lack of a convenient and sufficiently sensitive assay method.

We now report on a new radioenzymatic assay procedure, which is based on a two-step conversion of QUIN to $^3$H-deamido-NAD (d-NAD). In a non-reversible reaction, QUIN is first converted to nicotinic acid mononucleotide (NAMN) by a homogeneous preparation of quinolinic acid phosphoribosyltransferase (QPRT; cf. Köhler et al., this meeting). NAMN is further converted to d-NAD by a second enzyme, nicotinic acid mononucleotide adenyltransferase. In this step, $^3$H-ATP is used as a donor of the adenyl moiety, thus yielding $^3$H-d-NAD. In both enzymatic reactions, pyrophosphate appears as a side product. Addition of inorganic pyrophosphatase to the incubation mixture thus favors both the production of NAMN and the overall yield of d-NAD.

A batch procedure was found to produce optimal results. Enzymes, cofactors and co-substrates [$Mg^{2+}$ (1.5 mM), phosphoribosylpyrophosphate (0.3mM), $^3$H-ATP (0.1 µM)] and standards or tissue samples were incubated for 2 hours at 37°C in a total volume of 250 µl. Following termination of the reaction, $^3$H-d-NAD was separated from $^3$H-ATP using a Dowex-1x8 anion exchange resin (formate form) and quantitated by liquid scintillation spectrometry.

A linear standard curve was obtained between 2.5 and 250 pmol QUIN, with 2.5 pmol yielding values approximately 50% above blank. QUIN could be measured directly in 1 µl of human urine. The concentration in urine collected from six healthy normal males was 37.2 ± 7.3 µM. After deproteinization, QUIN was also determined in 50 µl aliquots of serum prepared from blood samples provided by the same six volunteers and its concentration found to be 0.41 ± 0.06 µM.

Adjustments are currently being made for application of this assay procedure for QUIN determination in tissue extracts. The development of a rapid, specific and inexpensive method for the accurate determination of QUIN can be expected to prove instrumental for the further assessment of both physiological and possible pathological roles of QUIN.

This work was supported by USPHS grants NS 16102 and 20509.

240.8 QUINOLINIC ACID PHOSPHORIBOSYLTRANSFERASE: PURIFICATION FROM RAT LIVER AND FIRST IMMUNOHISTOCHEMICAL STUDIES IN BRAIN TISSUE. C. Köhler, E. Okuno[1] and R. Schwarcz[1]. Dept. Pharmacology, ASTRA-Läkemedel, Södertälje, Sweden and [1]Maryland Psychiatric Research Center, P.O. Box 3235, Baltimore, MD 21228.

Quinolinic acid phosphoribosyltransferase (QPRT), the enzyme catalyzing the conversion of the endogenous excitotoxin quinolinic acid (QUIN) to nicotinic acid mononucleotide, has been identified in rat brain (J. Neurochem 44, 446, 1985). QPRT is a rate-limiting enzyme in the metabolism of tryptophan to NAD and may therefore regulate the intracellular levels of QUIN. This function, as well as the uneven regional and subcellular distribution of QPRT in the brain, suggested that further investigations of the characteristics of this enzyme may be of value for an understanding of QUIN-induced neurodegenerative phenomena.

Following a seven-step procedure, QPRT was purified 3600-fold, with a yield of 26%, from rat liver. The purified enzyme, subjected to polyacrylamide disc gel electrophoresis, migrated as a single protein band which coincided with QPRT activity. SDS electrophoresis yielded a single band corresponding to a molecular weight of 32,000 for QPRT subunits. Using gel filtration (Sephadex G-150) and sucrose density gradient centrifugation, the molecular weight of the enzyme was estimated to be 160,000.

Rat brain QPRT was partially purified (278-fold) using the initial purification scheme for the liver enzyme and its properties compared to those of the homogeneous liver preparation. Kinetic analyses, inhibition by phthalic acid ($K_i$=1.4 µM), physico-chemical properties and molecular weight determinations indicated the structural identity of QPRT from the two rat tissues. This was further confirmed immunologically, using antibodies raised against purified rat liver QPRT. The same antibodies were also employed to conduct immunohistochemical studies in rat brain. Specific QPRT immunoreactivity was found to be contained in small glial cell throughout the brain. Often, these cells were in close association with nerve cell bodies. Semiquantitative assessment showed pronounced regional differences in QPRT-positive cells, the densest areas being the olfactory bulb and circumventricular cells in the hypothalamus. Generally, the immunocytochemical picture thus parallelled biochemical analyses of QPRT activity. Antibodies raised against rat liver QPRT can therefore be expected to provide a useful tool for the detailed investigation of the brain's QUIN system (cf. also Okuno et al., this meeting).

This work was supported by USPHS grants NS 16102 and 20509 (to R.S.).

240.9 EFFECTS OF MPTP AND ITS METABOLITES ON NEUROTRANSMITTER DEGRADING AND SYNTHESIZING ENZYMES. C.W. Abell*, R.R. Fritz, N.T. Patel, R.-S. Shen, W. Gessner and A. Brossi. Div. of Biochemistry, Dept. of Human Biological Chemistry and Genetics, The University of Texas Medical Branch, Galveston, TX 77550 and The Medicinal Chemistry Section, Laboratory of Chemistry, NIADDK, NIH, Bethesda, MD 20205.

The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and a series of its derivatives were tested as substrates and/or inhibitors of monoamine oxidase B (EC 1.4.3.4) (MAO B), dihydropteridine reductase (EC 1.6.99.10) (DHPR) and tyrosine hydroxylase (EC 1.14.16.2) (TH). Initial rates of reaction for the oxidation of MPTP by pure human liver MAO B: antibody complex to its dihydropyridinium ($MPDP^+$) and pyridinium ($MPP^+$) metabolites were determined by measuring increases in absorbance at 340 nm and 290 nm, respectively. The $K_{m,app}$ and $V_{max,app}$ values for MPTP were 316 μM and 229 nmole/min/mg, respectively, while 4-phenyl-1,2,3,6-tetrahydropyridine (PTP) gave values of 221 μM and 196 nmoles/min/mg, respectively. $MPDP^+$ (0.2 mM), MPTP (1 mM) and $MPP^+$ (10 mM), but not PTP (1 mM), irreversibly inhibited MAO B.

Twenty-seven MPTP derivatives were tested as potential inhibitors of human liver and rat synaptosomal DHPR with q-6,7-dimethyl-4-hydroxy-dihydropteridine and NADH as substrates. MPTP, PTP, and the corresponding piperidine derivatives were found not to inhibit DHPR at lower than mM concentrations. All hydroxy derivatives inhibited DHPR noncompetitively and were 100-10,000 times more active than MPTP, and their inhibitory potencies increased with the number of hydroxyl substitutions present on the ring (catechol > phenol), and with the oxidation state of the nitrogen-containing ring (pyridine > tetrahydropyridine > piperidine).

Ten MPTP analogs were tested against rat striatal synaptosomes containing TH and partially purified rat pheochromocytoma TH using a $^{14}CO_2$-trapping microassay. MPTP, $MPDP^+$, $MPP^+$, and 1-methyl-4-(4'-hydroxyphenyl)-1,2,3,6-tetrahydropyridine did not inhibit TH at less than mM concentrations, but dihydroxylated derivatives of 4-phenylpyridine and $MPP^+$ were found to be the most potent mixed type inhibitors of TH ($K_i$ = 9.1 and 4.0 μM, respectively). Furthermore, the inhibitory potency of dihydroxylated compounds increased with the oxidation state of the N-containing ring (pyridine >> tetrahydropyridine > piperidine).

These results definitively demonstrate that pure human MAO B can oxidize MPTP to the reactive intermediate, $MPDP^+$. This compound and its oxidation product, $MPP^+$, are relatively weak inhibitors of DHPR and TH, but their hydroxylated derivatives are potent inhibitors at μM concentrations.

240.10 EFFECT OF DEBRISOQUIN ON BRAIN, CEREBROSPINAL FLUID AND PLASMA CONCENTRATION OF HOMOVANILLIC ACID AND 3-METHOXY-4-HYDROXY-PHENYLGLYCOL IN PRIMATES J. D. Elsworth,* D. E. Redmond, Jr. and R. H. Roth, Departments of Pharmacology and Psychiatry, Yale University School of Medicine, New Haven, Connecticut 06510

Debrisoquin is an antihypertensive drug which inhibits monoamine oxidase, but does not readily pass the blood-brain barrier. Its administration in man decreases plasma homovanillic acid (HVA) and 3-methoxy-4-hydroxyphenylglycol (MHPG) concentrations. In the monkey debrisoquin changes the arteriovenous concentration difference across the brain for MHPG, but not for HVA, indicating that somehow it may alter brain MHPG production without entering the brain. This study determines whether, in primates, debrisoquin administration alters actual brain tissue or CSF concentrations of HVA and MHPG. Such data are necessary to assess the validity of using the drug either to estimate the proportion of centrally derived HVA or MHPG present in plasma or to amplify a change in centrally produced HVA or MHPG measured in plasma. Even though there is evidence that peripherally produced MHPG may be under a degree of central control, there is interest in determining the origin of MHPG found in brain, CSF or plasma. MHPG measured in brain or CSF may be dependent to some extent on the concentration of blood-borne MHPG. By comparing the magnitude of decreases at these sites, the present study allows, in addition, an estimation of the influence that peripherally produced MHPG has on centrally measured MHPG.

Monkeys (Cercopithecus aethiops sabaeus) were treated by i.m. injection twice daily for one week with debrisoquin (0.3 mg/kg for 2 days, then 0.6 mg/kg for 2 days, then 1.5 mg/kg for 3 days). Samples of brain, CSF and plasma were taken 3 hrs after the last dose and their concentration of HVA and MHPG determined by gas chromatography-mass spectrometry and compared with values from untreated controls.

Statistically significant reductions were found in mean (± SE) plasma MHPG (70±3%) and plasma HVA (58±5%) concentrations. No change in HVA concentration was observed in CSF or brain. A statistically significant drop in CSF MHPG (39±9%) concentration occurred. Although mean MHPG concentration was lower in all 7 measured brain regions of the debrisoquin treated animals compared with controls, the reduction reached statistical significance only in cerebellum. The mean decrease in the 7 regions was 14±4%. Thus, the effect of debrisoquin on HVA production appears confined to the periphery; whereas, concomitant with a large decrease in plasma MHPG concentration, CSF MHPG was moderately reduced and that in brain slightly affected. This may be due to a limited contribution of peripheral MHPG to the central compartment and/or by an indirect action of debrisoquin on central MHPG production.

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## EXCITATORY AMINO ACIDS: GLUTAMATE AND GLUTAMATE ANALOGS

241.1 CHANGES OF THE BRAIN CONTENT OF QUINOLINIC ACID: POSSIBLE CORRELATION WITH BRAIN PATHOLOGY. F. Moroni, G. Lombardi* and V. Carlà.* Department of Preclinical and Clinical Pharmacology "M. Aiazzi Mancini"; University of Florence, 50134 Firenze, Italy.

Quinolinic acid (QUIN) is an excitotoxic tryptophan metabolite present in the brain of various animal species, including man. Possible changes of its brain concentration could be associated with neuropathology. In order to study how the brain content of QUIN is related to the tryptophan availability to the brain, its content was mass-fragmentographically measured in various brain areas of rats fed with tryptophan-free diet or in situations characterized by an abnormally high concentration of this amino acid in the blood. In the same brain areas the concentration of other tryptophan metabolites (5-hydroxytriptamine: 5HT; and 5-hydroxyindolacetic acid: 5HIAA) were also measured. In young adult rats fed with standard laboratory food the brain concentration of QUIN was: 1.5±0.04 (pmol/mg tissue) in the cortex; 0.4±0.05 in the cerebellum; 1.1±0.02 in the diencephalon; that of 5HT and 5HIAA were, respectively, 0.9±0.1 and 0.91±0.06 in the cortex; 1.98±0.12 and 2.45±0.25 in the caudate; 2.06±0.18 and 0.88±0.10 in the diencephalon. When rats were fed for 21 days with tryptophan-free diet (supplied by Piccioni, Brescia, Italy) the basic symptoms of pellagra appeared and the brain content of 5HT and 5HIAA markedly decreased (-70%). On the contrary, the cortical content of QUIN doubled. Thus, the rat brain seems able to synthetize QUIN utilizing metabolic pathways not always related to tryptophan and the possibility exists that this excitotoxin plays a role in the neuropathology of pellagra. On the other hand, tryptophan administration to rats resulted in a dose-dependent increase of the cortical content of 5HIAA and QUIN. Other experiments were performed in rats bearing a portocaval anastomosis, a situation characterized by a high blood content of aromatic amino acids. Under those conditions, the levels of 5HIAA and QUIN in various brain areas, increased by a factor of two, four weeks after surgery. This increase of 5HIAA and QUIN was also present in autoptically obtained human cortex derived from patients who died after hepatic coma. Thus, when the liver tryptophan metabolism is impaired, the brain content of QUIN increases. Therefore, this excitotoxin may be involved in the neurological pathology associated with liver diseases.

241.2 ASTROGLIAL REACTION TO INTRACRANIAL QUINOLINIC ACID INJECTION. H. Björklund*, L. Olson* and R. Schwarcz[1] (SPON: E.D. French) Dept. of Histology, Karolinska Institute, Stockholm, Sweden and [1]Maryland Psychiatric Research Center, Baltimore, MD 21228.

Astroglial reaction to intrastriatal and intrahypothalamic injections of the endogenous excitotoxin quinolinic acid (50 μg in 1 μl) was studied in adult rats, using immunohistochemistry with antiserum to glial fibrillary acidic protein. Animals were sacrificed 6 hours, 24 hours, 3, 7 and 30 days or 1 year after the injection. Six and 24 hours after quinolinic acid, the amount of glial fibrillary acidic protein-like immunoreactivity in the injected striatum was lower than in controls but returned to a normal level at 3 days. Not until 7 days was a clear striatal gliosis apparent, as evidenced by an increased density of glial fibrillary acidic protein-positive structures and brightly fluorescent, clearly hypertrophic cells. This gliosis was even more developed in animals sacrificed 30 days postoperatively. A weak astrocytic reaction was also observed in the ipsilateral corpus callosum at 6 hours after quinolinic acid. By 3 days, a marked gliosis, restricted to the injected hemisphere, was present throughout the corpus callosum, septum and cortex cerebri. In animals sacrificed 30 days after the quinolinic acid injection, the extrastriatal astrocytic reaction was clearly diminished, although the striatal gliosis was still prominent. One year post injection, no obvious gliosis could be observed in the cortex cerebri or the corpus callosum while striatal tissue, now markedly reduced in volume, was clearly gliotic. Using neurofilament antiserum, increased fluorescence intensity was noted in striatal nerve bundles during the first day after an intrastriatal quinolinic acid injection and persisted 1 year postoperatively.

In animals treated intrahypothalamically, a spherical central area almost devoid of glial fibrillary acidic protein-immunoreactivity was noted around the injection site 7 days after quinolinic acid administration. Around this area, gliosis was observed. Apart from a very restricted gliotic reaction around the needle track, no astrocytic reaction was observed in nicotinic acid injected control animals under any experimental condition.

We conclude that quinolinic acid causes both reversible and long-lasting gliosis when injected into the rat striatum. As a natural brain metabolite, quinolinic acid may constitute a particularly valuable tool for the elucidation of a possible role of glia in neurodegenerative disorders.

This work was supported by USPHS grants NS 16102 and 20509.

241.3 SIGNAL TRANSDUCING SYSTEMS FOR EXCITATORY AMINO ACID RECEPTORS: A MODEL STUDY ON CEREBELLAR GRANULE CELLS IN CULTURE. J. T. Wroblewski*, F. Nicoletti*, A. Novelli, A. Alho*, A. Guidotti and F. Costa (SPON: W. D. Blaker). Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hosp., Washington, D.C. 20032.

It may be expected that different subclasses of the excitatory amino acid receptors express their function by the activation of distinct coupling mechanisms associated with their recognition site. It has been demonstrated in various systems that excitatory amino acid agonists activate guanylate cyclase, increasing levels of cGMP. Our recent studies also indicate their functional role in promoting the hydrolysis of inositol phospholipids in slices of hippocampus, immature cerebellum and in cultured granule cells. Several lines of evidence indicate the possible coupling of excitatory amino acid receptors with increased conductance of $Ca^{2+}$ channels. The present study uses a primary culture of cerebellar granule cells obtained by 8-days old rats, consisting of an essentially homogeneous population of glutamatergic cells, showing a strong response to excitatory amino acids and devoid of the interference by the glial compartment. Our data indicate that the action of excitatory amino acid agonists results in the activation of two distinct types of $Ca^{2+}$ channels. Kainic acid strongly increases $Ca^{2+}$ uptake into granule cells through voltage-insensitive channels, since its action is not potentiated by veratrine or inhibited by tetrodotoxin. In contrast $Ca^{2+}$ uptake stimulated by classic NMDA receptor agonists strongly depends on the depolarization of the membrane indicating the involvement of voltage-sensitive $Ca^{2+}$ channels. The action of glutamate seems to indicate the occupancy of multiple receptor sites coupled both to voltage-sensitive and insensitive $Ca^{2+}$ channels. Similar differences among the above excitatory amino acid agonists are seen with respect to their ability to increase the intracellular levels of cGMP. However, different relations are observed when the hydrolysis of inositol phospholipids is monitored. This is especially pronounced in the action of quisqualic acid, which strongly stimulates inositol phospholipid hydrolysis, does not seem to increase intracellular levels of cGMP and behaves as a partial agonist of the kainic acid receptor with respect to $Ca^{2+}$ uptake. The results of the present and our previous work are summarized in a functional model describing the relations between the distinct recognition sites for excitatory amino acid receptor subtypes and intracellular responses involving increased calcium conductance, activation of guanylate cyclase and enhanced hydrolysis of inositol phospholipids.

241.4 EXCITATORY AMINO ACID RECEPTORS ARE COUPLED WITH INOSITOL PHOSPHOLIPID METABOLISM IN THE RAT CENTRAL NERVOUS SYSTEM. F. Nicoletti*, M. I. Iadarola, J. T. Wroblewski* and F. Costa (SPON: P. L. Canonico). Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hospital, Washington, D.C. 20032.

We have recently demonstrated that a variety of dicarboxylic amino acids, including ibotenic, quisqualic and homocysteic acids but not kainic acid or NMDA stimulate inositol phospholipid hydrolysis in the rat hippocampus. This was inferred from an enhanced accumulation of $^3$H-inositol-1-phosphate ($^3$H-I-1-P) in $Li^+$-treated slices prelabeled with $^3$H-inositol. This stimulation is selectively antagonized by the dicarboxylic amino acid receptor antagonist aminophosphonobutyric acid (APB). L-Glutamic and L-aspartic acids stimulate $^3$H-I-1-P formation by only 30% in hippocampal slices from adult rats but they markedly enhance (more than 15 fold) inositol phospholipid hydrolysis in hippocampal slices from newborn rats. In hippocampal slices from 6-7 day old animals, L-glutamic and L-aspartic acids are as potent as ibotenic acid and 4-7 fold more potent than norepinephrine and carbamylcholine in stimulating $^3$H-I-1-P formation. The action of L-glutamic and L-aspartic acids is markedly reduced in slices from 15 day old rats and is almost absent in slices from adult animals.

L-Glutamic and L-aspartic acids significantly enhance inositol phospholipid hydrolysis also in cerebellar slices from newborn animals and in primary cultures of cerebellar granule cells obtained from 8 day old rats but they fail to induce changes in $^3$H-I-1-P formation in cerebellar slices from adult rats. In primary cultures of cerebellar granule cells, the stimulatory action of L-glutamic acid on inositol phospholipid hydrolysis is antagonized by aminophosphonovaleric acid (APV) and $\gamma$-glutamylglycine but not by APB. This evidence suggests that the hippocampus and the cerebellum express two different molecular organizations of dicarboxylic amino acid receptors coupled with inositol phospholipid metabolism. The stimulation of inositol phospholipid hydrolysis by L-glutamic and L-aspartic acids or by other possible endogenous dicarboxylic amino acid receptor agonists may have an important role in the modulation of excitatory transmission as well as in the cellular mechanism(s) regulating neuronal growth and development.

241.5 CONTRIBUTION OF $Ca^{2+}$ TO NEURONAL EXCITATION ELICITED BY AMINO ACIDS: AN IN VITRO STUDY IN RAT PIRIFORM CORTEX. T.M. Galeno, N. Hori,* J.R. ffrench-Mullen,* and D.O. Carpenter. NY State Dept. of Health, Albany, NY 12201.

N-methyl-D-aspartate (NMA), quisqualic acid (Quis) and kainic acid (KA) have been shown to each activate a pharmacologically distinct receptor. However, the ionic mechanisms underlying activation of these receptors are unclear. On piriform pyramidal neurons, NMA elicits an apparent conductance decrease response whose magnitude increases with depolarization while Quis and KA elicit conductance increase responses whose magnitudes decrease with depolarization. The purposes of this study were to (1) determine, by the use of a $Ca^{2+}$ blocker, the contribution of divalent cations to excitation elicited by ionophoretically applied NMA, Quis and KA, and (2) determine the effects of varying $Ca^{2+}$ or $Mg^{2+}$ concentrations on neuronal sensitivity to these amino acids.

To determine if $Ca^{2+}$ contributes to the extracellularly recorded excitatory response elicited by NMA, Quis or KA, slices were perfused with a control Krebs-Ringer solution (phosphate and sulfate absent) followed by a 1.5 mM $Co^{2+}$ Krebs-Ringer solution. Responses to NMA, Quis and KA were reversibly blocked by $Co^{2+}$ perfusion. In separate experiments, cells perfused with zero $Ca^{2+}$ solutions showed enhanced responsiveness to NMA, Quis and KA compared to each respective control, with the KA response enhanced to the greatest degree. The potentiation of the KA response in zero $Ca^{2+}$ was often so great that attempts to elicit as many spikes in normal medium resulted in cell death. In 2X $Ca^{2+}$ solutions all responses were depressed. All responses were also sensitive to $Mg^{2+}$ but NMA was more sensitive than KA or Quis.

In spite of the difference in the apparent conductance produced by NMA as compared to KA and Quis, these results implicate $Ca^{2+}$ channels in all three responses. All are sensitive to the inorganic $Ca^{2+}$ channel blocker and the responses vary with external $Ca^{2+}$, although the KA response appears to be the most sensitive. In the absence of $Ca^{2+}$, the conductance of another ion, i.e., $Na^+$ may underly this excitation. The data is consistent with recent evidence that inward currents through the $Ca^{2+}$ channel are carried by monovalent cations when the external $Ca^{2+}$ concentration is reduced to a submicromolar level (Hess and Tsien, Nature 309: 453, 1984). The data are also consistent with the hypothesis that KA neurotoxicity is mediated at least in part by $Ca^{2+}$ entrance into the cell during KA receptor activation. There remains the dilemma of how to explain the differences in sensitivity to $Mg^{2+}$ and $Ca^{2+}$ and the differences in apparent conductance elicited by the different agonists.

241.6 CURRENT AND VOLTAGE CLAMP STUDIES ON THE MIXED AGONIST ACTION OF L-GLUTAMATE AND ITS ANTAGONISM BY D-AMINOPHOSPHONOVALERATE ON SPINAL MOTONEURONES IN VITRO.
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Glutamate (Glu), quisqualate (Quis) and N-methyl-D-aspartate (NMDA) are potent excitants of spinal motoneurones in vitro. Using intracellular recording from a frog spinal slice preparation we have attempted a direct demonstration of the selectivity of the antagonist D-aminophosphonovalerate (D-APV) against NMDA excitations compared to Glu and Quis.

In control Ringer ($Mg^{2+}$-free, 7°C) superfusion of 2 mM Glu, 30 µM Quis or 30 µM NMDA produced depolarizations of between 10 - 13 mV accompanied by modest conductance increases (20-30 %). Following prolonged superfusion (> 15 minutes) of varying concentrations (0.5 - 10 µM) of D-APV typically the NMDA responses were strongly antagonized while those to Glu were reduced with Quis relatively unaffected. For example 10 µM D-APV produced reductions of 100%, 39% and 10% for these amino acids respectively. A similar profile of antagonism was obtained in solutions containing 0.6 - 1.2 µM tetrodotoxin to abolish indirect effects via interneuronal activation.

Using the single microelectrode voltage clamp technique we have measured small slow inward currents produced in response to superfusion of low concentrations of Glu (1 mM) and Quis (15 µM). In 7 motoneurones maintained at resting potential level (mean -70 mV) in voltage clamp a net inward current of 103 pA was induced by Glu and 127 pA by Quis (6 cells). Characteristically the decay time for the Quis current (mean 106s) was prolonged compared with Glu (42s). D-APV (10 µM) reversibly reduced the Glu induced inward current by 33%, a value which corresponds well with the current-clamp data. In contrast the Quis induced currents were not antagonized (values were 108% of control) by D-APV.

This data confirms directly that D-APV is a selective and potent antagonist of NMDA excitation of motoneurones. The finding that D-APV antagonizes, albeit to a lesser extent, Glu responses of in vitro motoneurones confirms that Glu is a mixed agonist acting on quisqualate and NMDA receptors as described for cultured spinal neurones (Mayer and Westbrook, J. Physiol. Lond., 354: 29: 1984).

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241.7 RESPONSE PROPERTIES OF IDENTIFIED CORTICAL NEURONS IN TISSUE CULTURE TO EXCITATORY AMINO ACID AGONISTS AND ANTAGONISTS. J. E. Huettner and R. W. Baughman. Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115

Many neurons in the CNS are believed to use glutamate (Glu) or aspartate (Asp) as a transmitter and at least three types of Glu receptors have been distinguished pharmacologically. We have examined the responses of neurons from the visual cortex in dissociated tissue culture to a variety of Glu agonists and antagonists. In order to study the properties of a particular identified class of cortical neurons, we prelabeled cells in vivo by retrograde transport of a fluorescent tracer.

Dissociated cultures of neurons from the visual cortex of 7-10 day postnatal hooded rats were prepared as previously described (Huettner and Baughman. Soc. Neurosci. Abstr. 10:165 (1984)). Neurons in layer V that project to the superior colliculus were prelabeled in vivo by injecting fluorescent latex microspheres into the colliculus on postnatal day five. After 2-8 weeks in culture, neurons were impaled with intracellular microelectrodes, and amino acid agonists were applied by pressure ejection from a blunt micropipet. Antagonists were added to the perfusion medium and to the agonist pipet.

We have studied the responses of labeled layer V cells as well as the responses of other unlabeled cortical neurons to each of the following agonists: Glu (10-100uM), Asp(100uM), N-methyl-D-aspartate (NMDA, 10-100uM), kainate (1-10uM), and quisqualate (Quis, 0.1-1.0uM). All of these compounds produced rapid depolarizations in every neuron tested. The antagonist 2-amino-5-phosphonovalerate (APV, 20-50uM) completely blocked depolarizations due to NMDA but did not effect responses to kainate or Quis. Depolarizations due to 10-20uM Glu were diminished by APV but responses to higher concentrations of Glu (50-100uM) were not significantly reduced. Kynurenic acid (Kyn) and ϒ-D-glutamylglycine (ϒDGG, both at 250-1000uM) reduced responses to all of the agonists tested while glutamate diethylester at 500uM had no effect.

Our results indicate that the cortical neurons in our cultures posses NMDA receptors as well as receptors for kainate and Quis. In some neurons spontaneous and evoked excitatory post-synaptic potentials were observed, and these could be blocked by Kyn and ϒDGG. This finding suggests that the amino acid receptors described above are involved in excitatory synaptic inputs to these cortical neurons. (NIH EY03502)

241.8 DIFFERING EFFECTS OF GLUTAMATE ANALOGS AND ANTAGONISTS IN THE RABBIT RETINA. S.C.Massey*and R.F.Miller (SPON: A.I.Cohen). Ophthalmol. Dept.,Washington Univ.Sch.of Med.,St.Louis,MO 63110.

Glutamate, the putative neurotransmitter of photoreceptors and bipolar cells in the vertebrate retina, appears to activate a variety of post-synaptic receptors. These may be distinguished by their sensitivity to different glutamate analogs and, on this basis, have been classified as; 1)NMDA receptors,2)QQ receptors, 3)KA receptors and 4)APB receptors. The APB receptor in the retina appears to be uniquely associated with the ON bipolar cell and is not considered here.

In the outer retina, horizontal cells (HC) were directly depolarized by KA. QQ was less potent and NMDLA was ineffective. AP-7, a specific NMDA antagonist, had no effect but PDA and kynurenic acid (KynA), two general glutamate antagonists, hyperpolarized HC and blocked both the light response and the action of KA. These results are consistent with the dark release of an excitatory transmitter from photoreceptors which activates a KA (or QQ?) receptor on HC.

In the inner retina, KA and NMDLA both activate ganglion cells (GC) directly in the presence of $Co^{++}$. NMDLA typically causes burst-firing which is asspciated with small rhythmic oscillations in the membrane potential. Surprisingly, QQ had variable effects and sometimes excited, but more usually, inhibited GC. AP-7 specifically blocked the excitatory action of NMDLA but did not eliminate the light response or features such as directional selectivity. PDA blocked the light input to GC and the actions of KA and NMDLA, but not carbachol, thus demonstrating general specificity against glutamate analogs. KynA also blocked the light input, KA and NMDLA, but not the excitatory action of QQ. These observations suggest that the light driven input to GC is mediated mostly by KA receptors with a smaller component by NMDA receptors. The role of QQ receptors is unclear at this time, but these preliminary data are consistent with the possibility that independent QQ receptors are present on third order neurons of the retina.

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241.9 THE EFFECT OF $Mg^{+2}$ ON THE DESENSITIZATION OF THE GLUTAMATE ACTIVATED CURRENT IN ISOLATED FISH HORIZONTAL CELLS. T. O'Dell* and B.N. Christensen. Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX 77550.

Horizontal cells receive an excitatory synaptic input from photoreceptors that is thought to be mediated by glutamate (glu). In order to characterize the glutaminergic responses of horizontal cells, we utilized the whole cell voltage clamp variation of the patch clamp technique (Hamill et al., 1981) to measure current induced by bath application of glu in isolated horizontal cells enzymatically dissociated from catfish retina. Cells were voltage clamped at their resting membrane potential, and concentrations of glu ranging from 1-300 μM were superfused until a steady-state current was recorded.

At concentrations of <100 μM glu, the glu activated membrane current increased monotonically where it remained at a steady-state value for several minutes of superfusion time. There was no indication of desensitization. Application of glu by pressure ejection from a locally placed pipette produced a rapid increase in inward current similar to the response seen during bath application of glu. This shows that desensitization does not occur during the slower superfusion time period of bath application. When the glu concentration was increased to > 100 μM, the inward current reached a peak following which the current declined to a new steady-state level. This time dependent desensitization that occurs at elevated glu concentrations suggests either a low affinity binding site that reduces the channel conductance, or a block of the glu channel by the agonist.

The role of Mg in this desensitization process was investigated by comparing the response in normal extracellular Mg with that obtained from the same cell when Mg was removed. While the early portion of the current showed a peaking equal to that seen in normal Mg, the time-dependent desensitization was removed and the glu induced current remained at a level equal to the initial peak current. These results argue against a block of the glutamate channel and suggest instead that Mg may link the binding of glu to a low affinity binding site that reduces channel conductance.

Hamill et al., Pflugers Arch. 391:85-100.

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241.10 **INHIBITION OF $Ca^{++}$-DEPENDENT $^3$H-GLUTAMATE BINDING IN VIVO AND IN VITRO: A POSSIBLE MECHANISM FOR THE CYSTEAMINE-INDUCED SUPPRESSION OF KINDLING.** H.A. ROBERTSON, M.R. PETERSON* AND G.A. COTTRELL. Department of Pharmacology, Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7.

It has been suggested that cysteamine suppresses kindled seizures by depleting brain stores of somatostatin (Higuchi, T. et al., Brain Res., 288:359, 1983). However, cysteamine in the high concentrations used (200 mg/kg, i.p.) acts as a copper chelator and a free-radical scavenger and thus affects a wide variety of processes related to neurotransmission. Furthermore, somatostatin depletion and kindled seizure suppression do not follow the same time course (Cottrell and Robertson, this meeting). Another possible explanation for suppression of kindled seizures by cysteamine might be interference with excitatory neurotransmission. Glutamate appears to be the principle excitatory neurotransmitter in the brain. Accordingly, the effect of cysteamine on $Ca^{++}$-dependent glutamate binding sites was studied in vitro and in vivo.

Rats injected with saline or 200 mg/kg cysteamine were killed 24 hours after injection. A "buffy coat" membrane preparation was prepared from cerebral cortex and washed 5 times by centrifugation prior to final suspension in 50 mM Tris-HCl (pH 7.4). Specific binding of $^3$H-glutamate (20 nM) was determined in the presence and absence of 2 mM $Ca^{++}$. Non-specific binding was defined using 200 μM L-glutamate. Incubation at 37°C was terminated by rapid filtration on GF/B glass fiber filters.

Cysteamine inhibited $Ca^{++}$-dependent $^3$H-glutamate binding to cerebral cortical membranes both in vitro and in vivo. First, cysteamine (100 μM) incubated with membranes isolated from saline treated rats suppressed $^3$H-glutamate binding by 35%. Although this is a high concentration of cysteamine, it is within the range attained after systemic administration of 200 mg/kg of the compound. More significantly, however, when rats were given cysteamine (200 mg/kg i.p.) and were killed 24 hours later and $^3$H-glutamate binding to well-washed (5 times) cortical membranes assessed, cortical membranes from cysteamine-treated rats had significantly lower $Ca^{++}$-dependent $^3$H-glutamate binding (40%), even after the extensive washing necessary for glutamate binding studies.

These results suggest that interaction with glutamate binding sites is one possible mechanism by which cysteamine suppresses kindled seizures. It will also be necessary to determine whether cysteamine acts on other receptors and other aspects of neurotransmission. (Supported by the Canadian M.R.C.).

241.11 CHLORIDE ION DEPENDENT ASSOCIATION OF [3] L-GLUTAMATE WITH INTACT PC-12 CELLS. Charles E. Chandler* and E. Edward Mena (spon. C. J. Pazoles), Central Research Division, Pfizer Inc., Groton, CT 06340

Membrane binding assays for L-Glu are typically done using CNS membranes which are lysed and then washed several times in hypotonic medium. The membranes are then added to a defined ionic medium for assay. The binding sites measured in this manner exhibit a complex regulation by various ions. However, $Cl^-$ ions are responsible for the expression of the majority of L-Glu binding to CNS membranes (Mena et al., Brain Research, 243, 387, 1982). The effects of the lysing and extensive washing procedures on the expression of L-Glu binding sites is unknown. However, ionic gradients, which may occur if membranes reseal in the assay medium, could contribute to the ionic regulation of L-Glu binding.

We examined the association of [3] L-Glu (50 nM) with intact PC-12 cells. The cells were harvested with 150 mM Tris acetate (pH 7.4) and washed 3x (1000 xg for 5 min). The association of L-Glu with PC-12 cells was dependent upon the presence of $Cl^-$ ion in the external medium (ie. specific binding of 4933 ± 580 molecules/cell in 50 mM Tris HCl + 100 mM Tris acetate). The $Cl^-$ ion dependent association was saturable (Kd[Glu] = 2.78 µM) and was complete after 20 min at 30°C. Cells which were harvested and washed 3x in either 150 mM Tris HCl or 100 mM Tris HCl + 50 mM Tris acetate did not show any decrease in $Cl^-$ ion dependent association when assayed in 100 mM Tris acetate + 50 mM Tris HCl compared to cells harvested in 150 mM Tris acetate. Both quisqualic acid and ibotenic acid blocked this process (Ki values of 7.4 and 10.6 µM, respectively). However, 2-amino-4-phosphonobutyric acid (AP4) (100 µM) had no effect. Other compounds with no effect were (at 100 µM): N-methyl-D-aspartic acid, kainic acid, glutamic acid diethylester, alpha aminoadipic acid, quinolinic acid, kynurenic acid, AP3, AP5, AP6 and AP7.

Cells were not lysing during the assay since the release of cytoplasmic L-Glu would have raised the external concentration of 2-5 mM, a level over 1000-fold the Kd of the association. In addition, the cells were identical in appearance before and after the assay and only those cells which pelleted through 10% sucrose were recovered in the pellet.

The association of L-Glu with unlysed PC-12 cells has a requirement for $Cl^-$ ions in the external medium, is not affected by attempts to alter the internal/external $Cl^-$ ratios and exhibits rapid association kinetics. Thus, this $Cl^-$ dependent process occurs in intact cells as well as lysed CNS membranes.

241.12 SELECTIVE INHIBITION BY PHENCYCLIDINE ANALOGS OF N-METHYL-D-ASPARATE-EVOKED, BUT NOT KCl-EVOKED, [$^3$H]ACETYLCHOLINE RELEASE IN STRIATAL SLICES. S. McPherson*, P.L. Wood and J. Lehmann, Dept. of Neuroscience, CIBA-GEIGY Corp., Summit, NJ 07901 USA.

Lodge and co-workers have shown that phencyclidine and its analogs, as well as sigma opiate receptor agonists, preferentially counteract the excitation produced via stimulation of N-methyl-D-aspartate (NMDA)-type excitatory amino acid receptors (Berry et al., Br. J. Pharmac. 83:179, 1984). We report here that compounds active at [$^3$H]phencyclidine binding sites also inhibit the NMDA-evoked release of [$^3$H]acetylcholine (ACh), at concentrations well below those which inhibit KCl-evoked [$^3$H]ACh release.

At concentrations from 1 to 10 µM, greater than 50% inhibition of NMDA-evoked [$^3$H]ACh release was caused by phencyclidine, etoxadrol, ketamine, N-allyl-normetazocine, and cyclazocine. The 46% inhibition caused by N-allyl-normetazocine (1 µM) was not antagonized by haloperidol (100 nM), an antagonist of sigma opiate receptors, or by naloxone (100 µM), which antagonizes mu opiate receptors, among others (Su, JPET 223:284, 1982). The inhibition by N-allyl-normetazocine was not stereospecific, in agreement that this compound is not inhibiting NMDA-evoked [$^3$H]ACh release via sigma opiate receptors. The data are most consistent with an action at phencyclidine receptors mediating pigeon catalepsy and the binding of [$^3$H]phencyclidine (Mendelsohn et al., Biochem. Pharm. 33:3529, 1984), although the rank correlation is not perfect.

It can be excluded, however, that N-allyl-normetazocine is acting as a direct antagonist of NMDA-type receptors, since it fails to block lesions induced by intrastriatal injection of quinolinic acid, unlike the competitive NMDA-type receptor antagonists, 2-amino-5-phosphonopentanoate (AP5) and 2-amino-7-phosphonoheptanoate (AP7). Moreover, the slope for inhibition by phencyclidine of NMDA-evoked [$^3$H]ACh release is shallower than that of AP5 or AP7, suggesting a different mechanism of action.

Conclusions:

1. A receptor for phencyclidine and its analogs may be coupled to NMDA-type receptors, analogous to the benzodiazepine/GABA receptor/chloride ionophore complex.

2. This receptor, activated by sigma opiate agonists and phencyclidine and its analogs, is not the sigma opiate receptor, but may be the receptor labeled by [$^3$H]phencyclidine.

241.13 CHARACTERIZATION OF THE RESPIRATORY MOTOR RESPONSES TO PICOMOLE INJECTIONS OF EXCITATORY AMINO ACIDS INTO THE VENTRAL RESPIRATORY GROUP (VRG) OF CAT. D.R. McCrimmon*, J.L. Feldman, H.H. Ellenberger*, J.C. Smith* and D.E. Weese-Mayer* (SPON: C.H. Price). Department of Physiology, Northwestern University, Chicago, IL 60611

We have previously reported that small (0.2-10nl) injections of 1-10mM glutamic acid (Glu) or homocysteic acid (DLH) alters respiratory motoneuronal discharge pattern (Federation Proc. 44:428, 1985). In the current study, the responses to L-Glu and DLH were compared with those elicited by N-methyl-D-aspartic acid (NMDA). In addition, we tested the ability of relatively selective antagonists to block these responses and their effect on spontaneous phrenic nerve discharge. Experiments were performed on chloralose-urethane anesthetized, vagotomized, paralyzed and artifically ventilated cats. Phrenic nerve (5th cervical branch) discharges were recorded bilaterally. Pipettes of up to 7 barrels were used for extracellular unit recording and pressure ejection of precisely measured volumes of drugs; barrels contained the excitatory amino acids (1-10mM) Glu, DLH, and NMDA, the antagonists (1-100mM) 2-amino-4-phosphonobutyric acid (APB), glutamate diethyl ester (GDEE), and kynurenic acid (Kyn), and lidocaine (1-4%), GABA (10-100mM) and saline. Drugs were prepared in 150mM NaCl and adjusted to pH 7. At a given site within the VRG (where inspiratory modulated activity was recorded) the response to each of the excitatory amino acids was qualitatively similar but differed markedly in its magnitude and duration. Increases in VRG unit discharge and phrenic nerve discharge were elicited by injections into the lateral VRG 1 to 2mm rostral to the obex. Injections of as little as 250 femtomoles of L-Glu(0.25nl, 1mM) elicited marked transient increases in phrenic nerve discharge. Larger injections (2-4nl) lead to an alteration in the rhythm of phrenic nerve discharge. The order of potency was NMDA>DLH≥Glu. Decreases in phrenic discharge (consisting of transient apnea in many cases) were elicited by injections into the medial VRG from the level of the obex to about 2.5mm rostral. All 3 antagonists blocked both the excitatory and inhibitory responses with a potency Kyn>GDEE>APB. Injections of the antagonists themselves or lidocaine in amounts which were sufficient to block the responses to excitatory amino acids, had little or no effect on spontaneous phrenic nerve discharge. Similarly, injections of GABA (1-4nl, 100mM) inhibited unit discharge near the electrode tip but had no effect on spontaneous phrenic nerve discharge. These findings suggest that the excitatory amino acids elicit their effects by activating cells located in the VRG but which are not spontaneously active in this anesthetized preparation.

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242.1 CHARACTERIZATION OF NEURONAL DISCHARGE IN MEDIAL FRONTAL CORTEX WITH SPONTANEOUS CHANGES IN CARDIORESPIRATORY PARAMETERS ACROSS SLEEP-WAKING STATES. R.C. Frysinger, R.D. Frostig, V.L. Schechtman, J.D. Marks, and R.M. Harper. Brain Research Institute and Department of Anatomy, UCLA, Los Angeles, CA 90024.

Neurons in the medial frontal cortex discharge phasically with the cardiac and/or respiratory cycle, and this discharge is state dependent. The present study examines the relationship of cell discharge in this brain area with spontaneous changes in blood pressure and respiratory parameters across sleep-waking states.

Four adult cats were anesthetized with halothane and electrodes inserted for recording lateral geniculate nucleus and frontal cortex EEG, costal diaphragmatic and nuchal EMG, and eye movement. Microdrives were implanted to permit bundles of nine microwires to be lowered through the infralimbic cortex bilaterally. Arterial pressure was monitored continuously through a chronic femoral arterial cannula advanced into the descending aorta. Recording began 1 week following surgery, and up to five cells were simultaneously recorded along with the relevant cardiorespiratory and state assessment parameters.

Normalized discharge rates of 23 cortical cells have been correlated on a breath-by-breath basis with peak, minimal, and mean arterial pressure, and with $T_{TOT}$, $T_I$, and $T_E$ of the respiratory cycle. Separate analyses were done for each state for each cell, with each state period ranging from 100 to 600 seconds. Results from these analyses were then compared with standard cross-correlation histograms computed between these cells and onset of inspiration and the cardiac R wave.

Negative correlations between rate of discharge of cortical units and $T_{TOT}$ were observed in 17 cells, with ten of these showing a correlation in REM sleep. Two of these ten cells showed such a correlation in both waking and REM sleep states, but cells showing a correlation with $T_{TOT}$ in quiet sleep did not also correlate in REM. Phasic alterations in the discharge rate of four cortical units were associated with transient increases in systolic pressure during phasic events of REM sleep. There was no clear association between discharge timing relationships as revealed by the cross-correlation histogram and the correlation calculated between rate and cardiorespiratory parameters.

These results suggest a) cell discharge in medial frontal cortex is generally correlated with phasic events of REM sleep, and b) cortical cells showing discharge timing relationships to cardiac or respiratory cycles may not show tonic changes in discharge rate associated with fluctuation in cardiac or respiratory parameters.

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242.2 ROLE OF THE THALAMUS IN THE CONTROL OF SYMPATHETIC NERVE DISCHARGE. K.J. Varner*, S.M. Barman and G.L. Gebber. Dept. of Pharmacol./Toxicol., Mich. State Univ., East Lansing, MI 48824.

It is well known that patterns of neocortical activity such as the alpha (8-12 Hz) and delta (2-6 Hz) rhythms arise in the thalamus. The present study demonstrates that thalamic rhythm generating networks also exert influences on descending systems controlling sympathetic nerve discharge (SND). Experiments were performed on baroreceptor-denervated cats anesthetized with chloralose. Multicellular activity was recorded from the postganglionic inferior cardiac sympathetic nerve, medial thalamus (0-3 mm lateral to midline) and lateral thalamus. Crosscorrelation analysis revealed that a component of basal inferior cardiac SND was temporally related to the 2-6 Hz rhythm in thalamic activity. In some experiments, simultaneously recorded medial and lateral thalamic activity were sufficiently different so that SND was related only to medial thalamic activity. In other experiments, SND was related both to lateral and medial thalamic activity. That the temporal relationship between thalamic activity and SND was indicative of a functional connection is suggested by the results obtained with electrical stimulation. Single shocks applied to the medial thalamus elicited increases in inferior cardiac SND with onset latencies of 55-75 ms. Threshold responses could be elicited with a stimulus current as low as 70 μA. Sympathetic nerve responses evoked from the lateral thalamus had somewhat longer onset latencies. High frequency (50 Hz) stimulation of medial but not lateral thalamic sites produced marked increases in blood pressure. The increase in blood pressure elicited by medial thalamic stimulation was attenuated for approximately 10 min following the microinjection of 50 nl of 1 M L-glutamate at the site of stimulation. L-glutamate is believed to depolarize neuronal cell bodies but not axons. Thus, it appears that electrically-induced changes in blood pressure involved the activation of the somata of medial thalamic neurons.

Under the influence of subconvulsive doses of the GABA antagonist, picrotoxin, SND was temporally related to thalamic rhythmic activity in decorticate cats. Also, thalamic stimulation elicited increases in SND in decorticate cats in the absence or presence of picrotoxin. These results indicate the existence of a pathway from the thalamus to sympathetic nerves that does not include a cortical relay.

Although a 2-6 Hz rhythm persists in SND of the baroreceptor-denervated cat after decerebration, the period of the rhythm in some experiments is longer than that observed before decerebration. This result implies that brain stem sympathetic rhythm generating networks can be entrained by thalamic oscillators running at somewhat higher rates. If so, the leading focus for generation of the 2-6 Hz rhythm in SND may actually reside in the thalamus under certain conditions. (Supported by NIH grant HL13187.)

242.3 EXCITATORY NEUROTOXIN STIMULATED CARDIOVASCULAR RESPONSES FOLLOWING DIENCEPHALIC MICROINJECTIONS. R.W. Rockhold, C.-B. Jin*, C. Huang*. and J. Farley*. Dept. Pharmacology and Toxicology, Univ. Miss. Med. Cntr. Jackson, MS 39216

Kainic acid (KA), an excitatory neurotoxin which acts, in part, through stimulation of excitatory amino acid receptors, will produce increases in mean arterial blood pressure (MABP) and heart rate (HR) upon microinjection into brain regions in the vicinity of the paraventricular nucleus of the hypothalamus (PVN). The responses are dose-dependent (0.005-4.69 nmol) and accompanied by mydriasis, exopthalmos, increased respiratory rate and twitching of the vibrissae. Unilateral PVN injections (4.69 nmol in 100 nl) of KA, in urethane-anesthetized rats, increased MABP and HR by 61 ± 3 mm Hg and 114 ± 17 bpm, respectively; n = 10). Responses were observed within 30 sec following injections and persisted, at higher doses, for over 2 hr before returning to pre-injection levels. Pressor and tachycardic responses were selectively reversed by phentolamine (1 mg/kg, i.v.) and propranolol (1 mg/kg, i.v.) injections, respectively. Opthalamic, respiratory and motor responses were not altered by adrenoceptor antagonists. These data suggest predominantly sympathetic mediation of the cardiovascular responses. In support of this hypothesis, injection of a vasopressin vasoconstrictor antagonist (5 μg/kg, i.v.) lowered MABP by less than 10 mm Hg. Moreover, magnocellular neurons in the PVN were found to be largely intact upon light microscopic examination 3-7 days following unilateral PVN injections of KA (4.69 nmol) while parvocellular neuronal populations were reduced.

Responses observed following injections of N-methyl-D-aspartic acid (NMDA; 0.005-4.69 nmol) were similar but of much shorter duration (15-30 min). In contrast, L-glutamic acid produced only minimal increases in MABP and HR following unilateral PVN injections of 4.69 nmol.

Injections of KA or NMDA (0.469 nmol in 100 nl) into regions adjacent to the PVN, including the nucleus reuniens of the thalamus, the ventromedial nucleus of the hypothalamus and the anterior hypothalamus also increased MABP and HR.

The results indicate that neuronal soma in anteromedial hypothalamic and ventromedial thalamic regions possess excitatory efferent projections to cardiovascular regulatory areas.

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242.4 CARDIOVASCULAR EFFECTS OF PERFUSION OF THE ROSTRAL RAT HYPOTHALAMUS WITH CLONIDINE: DIFFERENTIAL INTERACTIONS WITH YOHIMBINE AND CORYNANTHINE. R.L. Commissaris*, F.C. Beuthin and D.K. Pitts*, Wayne State University, Detroit, MI 48202.

The present studies examined the interaction of two chemically-related alpha adrenoceptor antagonists on the cardiovascular effects of clonidine administered into the anterior hypothalamic/pre-optic (AH/PO) region of the forebrain by the push-pull perfusion technique. Push-pull cannulae were placed bilaterally into AH/PO region of anesthetized, paralyzed and ventilated rats. Perfusion of this area with artificial CSF (0.015 ml/min), yohimbine (5,50 uM) or its diastereoisomer corynanthine (50 uM) for 30 minutes did not affect mean arterial pressure or heart rate. Perfusion of the AH/PO region with clonidine (0.55 - 5.5 mM) resulted in a concentration-dependent reduction of mean arterial pressure and heart rate. The hypotensive effects of clonidine were found to be greater than the bradycardic effects. Co-perfusion with the alpha-2-adrenoceptor antagonist yohimbine (5,50 uM) significantly attenuated the hypotensive, but not the bradycardic, effects of a single concentration (1.75 mM) of clonidine; this selective antagonism of the hypotensive effect of clonidine by yohimbine was concentration-dependent. In contrast to yohimbine, the alpha-1-adrenoceptor antagonist corynanthine significantly attenuated the bradycardic, but not the hypotensive, effects of 1.75 mM clonidine. These results suggest that AH/PO clonidine perfusion depresses both mean arterial pressure and heart rate, and that the clonidine-induced hypotension is due to alpha-2-adrenoceptor activation, while the clonidine-induced bradycardia is due to alpha-1-adrenoceptor activation.

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242.5 DISTRIBUTION OF PEPTIDE AND SEROTONIN IMMUNOREACTIVE FIBERS AND TERMINALS IN PARAVENTRICULAR NUCLEUS OF THE CAT. M.M. Caverson, J. Ciriello, F.R. Calaresu and T.L. Krukoff. Department of Physiology, University of Western Ontario, London, Ontario, Canada N6A 5C1.

Recent evidence has shown that the paraventricular nucleus of the hypothalamus (PVH) consists of anatomically separate subdivisions which are involved in the regulation of neuroendocrine and autonomic responses. However, the distribution of biochemically distinct afferent projections to the different subdivisions of the PVH which may influence these responses has not been fully elucidated. In the present study the region of the PVH was investigated in cats for fibers and terminals immunoreactive to neurotensin, leucine-enkephalin, methionine-enkephalin, substance P, somatostatin and serotonin. Cats were perfused with Zamboni's fixative and 50 µm frozen transverse sections of the hypothalamus were cut and placed in antisera (Immuno Nuclear Corp.) for each of the neuroactive substances for 16-18 h at 4°C. The biotin-avidin immunohistochemical procedure (Vecta Stain Kit) was used. Fibers and terminal varicosities containing immunoreactivity for the six substances were found throughout the rostro-caudal extent of the region of the PVH as follows: 1) Neurotensin immunoreactivity had the greatest distribution throughout the PVH region. A dense terminal network was observed in the anterior periventricular area (Pet) which extended dorsolaterally into the ventral aspect of the dorsal component of the PVH (Pad) to form a cap over the fornix. Dense neurotensin immunoreactivity was also found in the ventral aspect of the anterior component of the PVH (Paa). 2) Leucine-enkephalin fibers and terminal varicosities were observed primarily in Pet and in Paa. A moderate density of labeling was found in the ventrolateral aspect of Pad and in the anterior aspect of the parvocellular nucleus (Pv). 3) The distribution of methionine-enkephalin immunoreactivity in the PVH region was similar to that of leucine-enkephalin, except that it was less dense. 4) Substance P, somatostatin and serotonin immunoreactivity had a similar distribution throughout the region of the PVH. Although fibers and terminal varicosities containing these substances were sparse, they were primarily concentrated in Pet, Pv and in the ventral aspect of Paa. These data indicate that specific neuroactive substances innervate different regions of the PVH and suggest that they may function in the regulation and co-ordination of neuroendocrine and autonomic responses.

(Supported by the Heart and Stroke Foundation of Ontario and MRC of Canada).

242.6 ACTIVATION OF SUPRAOPTIC VASOPRESSIN NEURONS BY STIMULATION OF RENAL AFFERENT FIBERS. T.A. Day and J. Ciriello, Department of Physiology, University of Western Ontario, London, Canada N6A 5C1.

Renal nerves are known to contain sensory afferent fibers which are thought to carry information from mechanoreceptors and chemoreceptors located in the kidney. Although activation of afferent renal nerves (ARN) have been shown to alter the firing frequency of hypothalamic cells (J. Autonom. Nerv.Syst. 3:311, 1981), it is not known whether ARN influence the discharge of identified magnocellular neurosecretory cells. The present study was done to determine whether information from the kidney was capable of altering the firing frequency of vasopressin (AVP)-synthesizing neurosecretory cells of the supraoptic nucleus (SON). Following transpharyngeal exposure of the hypothalamus and hypophysis, extracellular recordings were obtained from SON neurosecretory cells identified by antidromic invasion from the neurohypophysis. The effect of electrical stimulation (3-5 pulses, 75-300 Hz, 0.1-1 ms pulse duration) of the central end of severed nerves isolated from the left renal plexus on these cells was determined. Fifty-three spontaneously active neurosecretory cells were tested. As previously described (J. Physiol. 355:237, 1984), these units were classified as AVP or oxytocin (OXY) secreting on the basis of basal activity patterns and response to baroreceptor activation. All contralateral (n=14) and 69% of ipsilateral (total n=26) putative AVP cells tested were excited by stimulation of ARN. Mean latency to onset of excitation was 189 ± 5 ms, suggesting a total afferent pathway conduction velocity of less than 1 m/s. Mean duration of the excitation was 237 ± 17 ms. Stimulus pulse durations of less than 0.3 ms were ineffective in eliciting responses from putative AVP cells. In combination with the low afferent pathway conduction velocity, this was interpreted to suggest that the afferent fibers involved were unmyelinated. Thirteen of the 53 units examined were identified as putative OXY neurons; none of these cells showed any response to stimulation (5 ipsilateral, 8 contralateral) of ARN.

AVP, released into the bloodstream from the neurohypophysis, is known to regulate renal water excretion and exert a significant influence upon general cardiovascular function as a consequence of its vasoconstrictor properties. The present data suggest that afferent signals originating in the kidney exert a stimulatory effect on the activity of AVP neurosecretory cells which is likely to contribute to the regulation of circulating plasma levels of this hormone and the level of arterial pressure.

(Supported by the Heart and Stroke Foundation of Ontario).

242.7 NORADRENERGIC MODULATION OF SUBFORNICAL ORGAN INPUT TO THE NUCLEUS MEDIANUS. C.A. Graham*, D.O. Nelson, and A.K. Johnson. Department of Physiology, Northwestern Univ. Med. Sch., Chicago, IL 60611.

The subfornical organ (SFO) is an important neuronal structure mediating cardiovascular and behavioral responses to systemic angiotensin II (AII). SFO projections include the nucleus medianus (NM). Our recent in vitro investigations indicate that this projection is excitatory and may contain AII. Recent neuroanatomical studies have demonstrated heavy ascending noradrenergic projections to the NM which, based on chemical lesion studies, are necessary for mediation of AII-evoked drinking behavior. This study investigated electrophysiologically the role of noradrenergic input on NM neurons receiving SFO input utilizing the in vitro brain slice technique. Sagittal brain slices (400um thick) of the anterior ventral third ventricle (AV3V) were prepared from rats. This preparation allowed direct visualization of the SFO and NM. Spontaneous NM neuronal activity was recorded through one barrel of a multibarrel micropipette. Additional barrels contained norepinephrine, AII, or Saralasin and were connected to a custom micropressure injection system providing 100pl resolution. Low frequency electrical stimulation of the SFO produced short latency exciation of 60% (n=31) of the spontaneously active cells located in the NM. Addition of norepinephrine directly to the perfusing medium (1uM-100uM) or by simultaneous micropressure application (0.2-5nl) markedly enhanced the excitatory response of 80% of NM neurons responding to SFO stimulation. Exposure to norepinephrine reduced spontaneous ongoing activity of most NM neurons. Addition of Saralasin (0.1uM) to the perfusing medium or by direct micropressure application reversibly blocked or attenuated neuronal responses to SFO stimulation. Most of the NM cells were also excited by direct application of AII. Direct activation by AII was also antagonized by simultaneous pressure application of Saralasin. Saralasin alone reduced spontaneous activity in many cases. In a small number of cases (n=8), the enhancing effects of norepinephrine were blocked by application of Saralasin.

Our results indicate that noradrenergic input to NM neurons may enhance neuronal sensitivity to SFO input. Convergence of noradrenergic input and angiotensinergic pathways from the SFO on NM neurons may exist. Current studies are focusing on receptor populations mediating these effects.

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242.8 A [$H^3$] 2-DEOXYGLUCOSE MAPPING OF CENTRAL PATHWAYS MEDIATING THE PRESSOR RESPONSE TO ELECTRICAL STIMULATION OF THE SUBFORNICAL ORGAN IN THE RAT. M.B. Gutman*, J. Ciriello and G.J. Mogenson (SPON: M.A. Cook). Dept. of Physiology, Univ. of Western Ontario, London, Canada N6A 5C1.

The subfornical organ (SFO) has recently been shown to be a primary site at which bloodborne angiotensin II activates a neural circuitry associated with drinking behavior and increases in arterial pressure, heart rate and vasopressin secretion. However, the central structures that are part of this neural circuit have not been completely elucidated. The present study was done to identify structures in the central nervous system which are activated by electrical stimulation of pressor sites in the SFO. Experiments were done in sinoaortic denervated rats anesthetized with urethane. Tritiated 2-deoxyglucose (2DG) was administered intravenously while the SFO was electrically stimulated (15 Hz, 150 µA, 1.5 ms pulse duration, 45 min pulse train). Control animals underwent the same surgical procedure, including the placement of an electrode 1.5 mm dorsal to the SFO and then received the same dosage of 2DG one minute after initiation of a 45 minute infusion of phenylephrine (PE) (5-10 µg/kg/min i.v.) and no electrical stimulation. Mean arterial pressure was increased by 37 ± 2 mm Hg during stimulation of the SFO, which was similar to the rise in arterial pressure observed during PE infusion in control animals (37 ± 13 mm Hg). After the 45 minute stimulation or infusion period the brain and thoracic spinal cord was quickly removed and frozen. Transverse sections were cut at 20 µm, quickly dried on cover glass and placed in contact with tritium sensitive film for 8 weeks. Autoradiographs were developed and analyzed for areas having changes in metabolic activity as indicated by the density of photographic emulsion. Increases in density in experimental animals compared to controls were observed in the regions of the nucleus accumbens, the dorsal and ventral components of the nucleus medianus, the septofimbrial nucleus, the nucleus triangularis, the lateral septum, the rostral suprachiasmatic nucleus, the dorsal thalamus, the supraoptic nucleus, the paraventricular nucleus of the hypothalamus, the medial habenula and the intermediolateral nucleus of the thoracic cord. These data have demonstrated that stimulation of pressor sites in the SFO increases the 2DG uptake of several forebrain and spinal cord structures which are likely involved in the control of body fluid balance and arterial pressure.

(Supported by Heart and Stroke Fdn. of Ontario and MRC of Canada).

242.9 **EFFERENT CONNECTIONS OF THE MEDIAL PREOPTIC REGION IN THE RAT.** E.R. Brown*, D.G. Standaert* and C.B. Saper. Depts. of Neurology and Anatomy and Neurobiology, Wash. Univ. Sch. of Med., St. Louis, MO 63110

The medial preoptic region, surrounding the anteroventral third ventricle (AV3V), is involved in the regulation of blood volume, pressure, and composition. In order to better understand the mechanism for this control, we studied the efferent pathways from this area in rats using anterograde transport of tritiated amino acids, as well as a new lectin tracer, PHA-L. Two cell groups were found to project to structures which have been implicated in cardiovascular control: the median preoptic nucleus (MnPO), and the anteroventral periventicular nucleus (AVPV). The projections of these cell groups are similar. One group of descending fibers takes a periventricular route, and innervates both the endocrine and autonomic regulatory portions of the paraventricular nucleus of the hypothalamus. Other fibers innervate the arcuate and periventricular nuclei. Some turn ventrolaterally to run into the supraoptic nucleus, and others extend dorsally to innervate the subfornical organ. The remaining periventricular fibers continue caudally into the periaqueductal grey matter, and some can be traced as far as the dorsal lateral parabrachial nucleus, and, after AVPV injections to the nucleus of the solitary tract and the ventrolateral medulla.

A second group of descending fibers runs dorsolaterally from MnPO and AVPV into the bed nucleus of the stria terminalis, innervating the ventral and lateral parts of the nucleus. Some fibers turn caudally, following the column of the fornix into the lateral hypothalamic area. At the premamillary level, these fibers sweep dorsomedially to join those running from the periventricular system into the periaqueductal gray matter. We have recently found that many of the neurons in AVPV stain immunohistochemically for atrial natriuretic peptide (atriopeptin). Atriopeptin immunoreactive fibers are also found in most of the fiber pathways we have described from AVPV to cell groups involved in central cardiovascular control.

These projections, and those from MnPO, may underlie many of the cardiovascular phenomena observed following stimulation or lesions of the AV3V region.

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242.10 **ORIGIN OF ATRIOPEPTIN-IMMUNOREACTIVE INNERVATION OF THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS (PVH).** D.G. Standaert*, E.R. Brown*, P. Needleman*, and C.B.Saper. Depts. of Pharmacology, Neurology, and Anatomy and Neurobiology, Wash. Univ. School Med., St. Louis, MO 63110. (SPON: J. Trotter)

Atrial natriuretic factor, or atriopeptin, is a recently described peptide hormone, found within secretory granules in the myocytes of the cardiac atria, that has natriuretic and diuretic properties. We have used antisera raised against synthetic atriopeptin III and a cyanogen bromide fragment of atriopeptigen, the high molecular weight precursor of atriopeptins, to identify atriopeptin-immunoreactive (AP-ir) neurons in the brain of the rat.

The most prominent collection of AP-ir neurons is found in the anteroventral periventricular nucleus (AVPV), adjacent to the tip of the third ventricle. Inspection of the pattern of fiber staining suggests that this nucleus is the primary source of a dense collection of AP-ir fibers that innervate PVH.

We have used several different neuronal tracing techniques to study in detail the AP-ir innervation of PVH. Injections of WGA-HRP into PVH demonstrate extensive retrograde labelling of neurons in AVPV. Very small ionotophoretic injection of PHA-L, an anterograde tracer, into AVPV reveal a dense network of fibers ramifying within the magnocellular and parvocellular parts of PVH. Fluorescent retrograde tracers in combination with immunohistochemistry were used to identify the origin of AP-ir innervation to PVH. Injections of Fast Blue or diamidino yellow into PVH demonstrated that many of the AP-ir cells in AVPV send projections to PVH. In addition, a small number of the AP-ir neurons located in the lateral dorsal tegmental and pedunculopontine nuclei contribute to the AP-ir terminal field in PVH.

Both AVPV and PVH play an important role in the regulation of cardiovascular function. The AP-ir projection from AVPV to PVH may represent the anatomical substrate for interaction between these two nuclei. Thus, atriopeptin may function as a central neurotransmitter as well as a peripheral hormone in the regulation of the cardiovascular system.

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242.11 **RELATIONSHIPS OF VISCERAL AFFERENT AND LIMBIC CONNECTIONS IN THE INSULAR CORTEX IN THE RAT.** D.F.Cechetto and C.B.Saper. Depts. of Neurology and Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO, 63110.

The presence of a vagal representation in the insular cortex (IC) of the rat and cat has been established both by neuroanatomical and neurophysiological methods. In addition, limbic projections from the amygdala and the medial dorsal nucleus of the thalamus (MD) may converge with visceral afferents in this area. In the present experiments we have investigated the anatomical relationship in the IC of visceral afferent and limbic representations. The IC was explored in 19 rats for units with responses to visceral inputs. HRP was iontophoretically injected at the recording site of one visceral responsive unit in each animal. A viscerotopic organization was observed, in which units in the rostral part of granular IC received taste and gastromechanoreceptor inputs while cardiopulmonary inputs projected to the caudal part of granular IC. Retrograde HRP label indicated that the taste area in IC received afferents from the ventroposteromedial and ventromedial basal nuclei of the thalamus and the rostral part of the medial parabrachial nucleus (PB). The cardiopulmonary area of IC received input from the ventroposterolateral nucleus of the thalamus. However, most of the latter the sites were dorsally located in granular IC. Injections of WGA-HRP in PB showed that the projections to posterior IC were more ventrally located than those to anterior IC. None of the visceral receptive sites showed connections with the amygdala or MD, but WGA-HRP injections into these areas resulted in labeling that was primarily restricted to the agranular IC, which was ventral to all of our recording sites.

In summary, IC appears to be composed of a series of longitudinally oriented overlapping strips including: a) a dorsal granular IC area receiving spinal and trigeminal visceral-related input in a viscerotopic manner, b) a ventral granular and dorsal agranular IC region receiving vagal inputs, and c) a ventral strip including the ventral agranular and ventral part of the dorsal agranular IC, which receives amygdaloid and MD inputs.

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242.12 AORTIC BARORECEPTOR DENERVATION ALTERS REGIONAL HEXOKINASE ACTIVITY IN THE FOREBRAIN OF THE RAT. W.E. Turton*, F.R. Calaresu and J. Ciriello. Dept. of Physiology, Univ. of Western Ontario, London, Canada N6A 5C1.

It has been demonstrated that selective denervation of aortic baroreceptors in the rat results in a persistent elevation in arterial pressure (AP). Recently, it has been shown that the paraventricular nucleus of the hypothalamus (PVH) is involved in both the development and maintenance of the elevated AP in this model of experimental hypertension. However, little is known about other central structures that may be associated with the hypertensive process. In the present study, those regions of the brain whose metabolic activity is altered after aortic baroreceptor denervation were functionally mapped using hexokinase (HK) histochemistry, which reflects the rate of glucose utilization in the brain. After recording AP (tail cuff method) and heart rate (HR) for a control period of 4 days, rats were subjected to either bilateral transection of the aortic depressor (ADN) and cervical sympathetic nerves or to sham-ADN transection. Three days after ADN surgery, rats were anesthetized with sodium pentobarbital and perfused transcardially with warm saline. The brains were removed and quickly frozen, sectioned (20 μm) on a cryostat and mounted on glass slides. Transverse forebrain sections were processed for HK histochemistry as described previously (Acta Histochem., 28:286, 1967). Changes in HK activity were assessed by densitometric measurements of areas containing HK reaction product. AP and HR were elevated by 26 ± 4 mmHg and 50 ± 17 bpm, respectively, in the ADN-transected animals compared to sham-ADN transected animals. Significant increases in HK activity were observed in the subfornical organ, in the magnocellular and parvocellular components of the PVH, and in the supraoptic nucleus of the ADN-transected animals. These data have demonstrated that removal of aortic baroreceptor afferent inputs alters the activity of forebrain structures previously implicated in the regulation of body fluid balance and arterial pressure and suggest that these structures are involved in the hypertensive process after ADN transection.

(Supported by Heart and Stroke Fdn. of Ontario and MRC of Canada).

243.1 BRAIN Na,K-ATPase: EVIDENCE FOR MEMBRANE ADAPTATIONS TO CHRONIC ETHANOL AND NORADRENERGIC STIMULATION. A.C. Swann, Department of Psychiatry, University of Texas Medical School, Houston, TX. 77225

We have previously shown that the effects of chronic ethanol on Na,K-ATPase were opposite to those of ethanol in vitro, consistent with a membrane adaptation to the membrane-disordering effect of ethanol (JPET 232:475-479, 1985). There is evidence in vitro that ethanol prevents metabolic effects of norepinephrine (NE) related to Na,K-ATPase stimulation and that norepinephrine increases the sensitivity of Na,K-ATPase to inhibition by ethanol. Exposure to norepinephrine in vivo appears to regulate the number of Na,K-ATPase sites (Brain Res. 260:338-341, 1983). We therefore examined the interactions between ethanol and noradrenergic stimulation in vivo. Repeated noradrenergic stimulation (yohimbine 2 mg/kg bid for one week) reduced the K+ affinity, increased the affinity for ATP, and increased both $\Delta H$ and $\Delta S$ for K+ binding and for the rate-limiting conformational change of Na,K-ATPase. Sensitivity to inhibition by ethanol in vitro and the enhancement of ethanol inhibition by norepinephrine were increased by repeated yohimbine. Repeated yohimbine also reduced the Hill coefficient for inhibition by fluoride ion. These effects were all consistent with increased membrand fluidity, similar to effects of ethanol in vitro and opposite to chronic ethanol. Combined chronic ethanol and yohimbine had effects that were generally intermediate between those of either alone. Depletion of norepinephrine with DSP4 had essentially the opposite effects. Chronic ethanol reduced both the increase in Na,K-ATPase after repeated yohimbine and the decrease after DSP-4. These results suggest that repeated noradrenergic stimulation may affect membrane fluidity in the region of Na,K-ATPase in a manner that alters 1) the coupling of cation transport to energy supply and 2) the response to ethanol. Ethanol appeared to reduce the sensitivity to norepinephrine in vivo.

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243.2 PLASMA EPINEPHRINE AND NOREPINEPHRINE DURING CPR IN DOGS. P.J. Foley*, W.A. Tacker*, W.D.Voorhees*, M.A. Elchisak. Biomedical Engineering Center and Dept. of Physiology and Pharmacology, Purdue University, W. Lafayette, IN 47907.

Epinephrine is frequently given during advanced life support for patients with sudden cardiac death syndrome. However, the role of endogenous catecholamines in survival is not known. To determine the sympathomedullary response to cardiac arrest, plasma epinephrine (E) and norepinephrine (NE) levels were measured before, during and after cardiac arrest. Twelve mongrel dogs weighing 20 to 25 kg were anesthethized with pentobarbital sodium (30 mg/kg, i.v.), intubated and catheterized for the following pressures: left ventricular (LVP), aortic (AP) and central venous (CVP). Ventricular fibrillation was induced electrically. Standard American Heart Association CPR was performed using a pneumatically driven device (Thumper®). CPR was standardized in all dogs by adjusting the force of compression to establish and maintain an end-diastolic arterial-venous pressure gradient of 20 to 25 mmHg. No exogenous catecholamines were given. Endogenous plasma E and NE were determined, using HPLC, from blood samples drawn from the CVP catheter before cardiac arrest and at 2.5, 4.5, 9.5 and 11.5 min. during CPR. After 12 min. of CPR, the cardiac ventricles were electrically defibrillated and the dog was allowed to recover. Blood samples were drawn at 5, 10, 15, and 20 min. post-resuscitation. Plasma E and NE increased from prearrest levels of 28.27±4.38 (±SEM) and 152.52±16.39 pmoles/ml to 358.55±75.75 and 280.79±47.90 pmoles/ml, respectively, at 2.5 min. of arrest. E levels peaked at 380.40±54.84 pmoles/ml at 4.5 min. of CPR and fell to 52.79±13.34 pmoles/ml at 20 min. post-resuscitation. NE levels peaked at 1140.73±123.70 pmoles/ml at 11.5 min. of CPR and fell to 472.87±68.07 pmoles/ml at 20 min. post-resuscitation. It is concluded that cardiac arrest causes significant increases in plasma E and NE levels which remain elevated for the duration of the the arrest. After resuscitaion the plasma E and NE levels decrease gradually toward control values. These results are similar to those reported in human patients during CPR.

This work was carried out under a Fellowship Grant from the American Heart Association, Indiana Affiliate, Inc.

243.3 CHARACTERIZATION OF THE ISOZYMES OF PHENYLETHANOLAMINE N-METHYLTRANSFERASE. D.L. Wong, L. Yamasaki* and R.D. Ciaranello. Dept. of Psychiatry and Behavioral Sciences, Stanford Univ. Sch. of Med., Stanford, CA 94305

Phenylethanolamine N-methyltransferase (PNMT) has been purified to homogeneity by the criteria of high sensitivity silver staining of protein analyzed on 12.5 percent sodium dodecylsulfate polyacrylamide gels. The enzyme was purified from bovine adrenal medulla by ammonium sulfate precipitation, acid precipitation, Sephadex G-75 chromatography and affinity chromatography on S-adenosylhomocysteine (SAH)-Sepharose 4B. Isoelectric focusing of the purified enzyme shows that there are at least five isozymes with isoelectric points ranging from 4.5 to 5.5. Enzymatic activity is associated with each isozyme as shown by activity assay of gel slices. Each isozymic species is immunoreactive as determined by immunoblotting and immunofixation using a polyclonal antibody generated against the purified enzyme. This PNMT antibody does not cross-react with dopamine ß-hydroxylase (DBH). Similarly, the PNMT isozymes do not cross-react with polyclonal DBH antibody. Treatment of PNMT with various endo- and exoglycosidases suggests that the differences between isozymes may be partially due to carbohydrate constituents. The carbohydrate components consist of high mannose and complex sugars covalently linked through diacetylchitobiose to an asparagine residue in the polypeptide chain. $K_m$'s for the substrate, norepinephrine, and the cofactor, S-adenosylmethionine, are being determined for each isozyme. Polypeptide mapping of limited proteolytic digests of the deglycosylated isozymes is being used to determine whether any variations in primary structure exist.

243.4 ELECTROPHYSIOLOGICAL EFFECTS OF COCAINE IN THE MESOACCUMBENS AND MESOCORTICAL DOPAMINE SYSTEMS. F.J. White. Dept. Psychology, Univ. of Illinois, Champaign, IL 61820.

Despite the increasing frequency of cocaine abuse, surprisingly little is known about the effects of this psychomotor stimulant on the activity of individual CNS neurons. Although biochemical techniques have shown that cocaine blocks the high affinity uptake of monoamines, the relationship of this effect to the activity of individual neurons remains to be established. Because cocaine's rewarding effects are known to involve the mesolimbic and mesocortical dopamine (DA) systems originating in the ventral tegmental area (VTA) and projecting to various forebrain sites including the nucleus accumbens (NAc) and prefrontal cortex, the present experiments used single unit recording and microiontophoretic techniques to determine the effects of cocaine on the electrical activity of these DA systems.

Intravenous (i.v.) cocaine administration to chloral hydrate anesthetized rats caused a dose-dependent, haloperidol-reversible reduction in the firing of antidromically identified mesoaccumbens DA neurons but failed to alter the activity of mesocortical DA neurons. The ID50 for suppression of the inhibited neurons was 2.96 ± 0.86 mg/kg and was negatively correlated with the basal activity of the neurons. The extent of suppression was seldom greater than 60%, even at high doses. Microiontophoretic administration of cocaine (0.1 M, 60 nA) caused a weak suppresssion of the majority of A10 DA neurons; this effect was also correlated with the basal rate of the cell. At higher currents, decreases in splike amplitude were usually present suggesting a local anesthetic action. The inhibitory effects of cocaine were blocked by coiontophoresis of l-sulpiride (0.05 M). At lower currents (10-20 nA), cocaine significantly increased and prolonged the inhibitory effect of iontophoretic DA (0.01 M) as well as the inhibition caused by antidromic stimulation of the NAc. Recordings from single units within the NAc revealed that cocaine also significantly potentiated the inhibitory effects of both iontophretic DA and electrical (pulse-train) stimulation of the VTA.

These experiments have shown that cocaine can inhibit the activity of mesoaccumbens A10 DA neurons as well NAc cells which receive a DA innervation from the VTA. The mechanism responsible for such inhibition appears to be that of uptake inhibition since cocaine potentiates the effects of both iontophoretic DA and electrical stimulation of the DAergic fibers. These results also provide further confirming evidence that the mesocortical DA neurons lack impulse-regulating DA autoreceptors since these neurons failed to respond to i.v. cocaine administration.

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243.5 LATENT NORADRENERGIC NEUROTRANSMISSION IS STIMULATED BY β-PHENYLETHYLAMINE IN RAT HIPPOCAMPAL SLICES. S. Arbilla*, S. Benkirane and S.Z. Langer, Department of Biology, Laboratoires d'Etudes et de Recherches Synthélabo (L.E.R.S.), 58, rue de la Glacière, 75013 Paris, France

The stimulation-evoked release of $^3$H-5HT from rat cerebral slices is modulated by inhibitory $\alpha_2$-adrenoceptors (Langer S.Z. and Moret C., J. Pharmac. Exp. Ther. 222 : 220, 1982). We have used the $\alpha_2$-adrenoceptor mediated modulation of the electrically-evoked release of $^3$H-5HT from rat hippocampal slices as a model to evaluate the in vitro influence of β-phenylethylamine (β-PEA) on noradrenergic neurotransmission.

The slices were labelled with $^3$H-5HT and perfused in a Krebs medium. The calcium-dependent release of $^3$H-5HT was elicited by two periods of electrical stimulation ($S_1$ and $S_2$) at 3 Hz, 24 mA for 2 min. Provided that monoamine oxidase B was inhibited by deprenyl 1 μM, exposure to β-PEA (1 - 10 μM) added 20 min before $S_2$ inhibited the electrically-evoked release of $^3$H-5HT in a concentration-dependent manner. In the presence of β-PEA 3 μM, the ratio $S_2/S_1$ was : 0.53 ± 0.03, $p < 0.001$, n = 11 ; (control : $S_2/S_1$ = 0.83 ± 0.03, n = 8). Under the same experimental conditions, 6-F-NA 0.1 μM significantly inhibited $^3$H-5HT release ($S_2/S_1$ = 0.34 ± 0.02, n = 5, $p < 0.01$). Idazoxan 1 μM added before $S_1$ antagonized the inhibitory effect of β-PEA 3 μM ($S_2/S_1$ = 0.90 ± 0.05, n = 9) (control in the presence of idazoxan : $S_2/S_1$ = 0.99 ± 0.04, n = 9). Inhibition of tyrosine hydroxylase (TH) activity by α-methyl-p-tyrosine (300 mg/kg i.p., 2 h + 100 μM in the Krebs medium) did not affect the inhibition by 6-F-NA 0.1 μM of $^3$H-5HT release ($S_2/S_1$ = 0.34 ± 0.07, n = 4, $p < 0.001$), but it antagonized the inhibitory effects of β-PEA 3 μM ($S_2/S_1$ = 0.89 ± 0.13, n = 4). The antagonism of the effects of β-PEA by the selective $\alpha_2$-adrenoceptor blocker idazoxan indicates the involvement of these receptors in the inhibition by β-PEA of $^3$H-5HT release. The lack of effects of β-PEA on the release of $^3$H-5HT after inhibition of TH activity indicates that β-PEA acts indirectly on $\alpha_2$-adrenoceptors through the release of newly synthesized noradrenaline, namely by promoting a latent noradrenergic neurotransmission in a similar manner as it was shown for amphetamine on dopaminergic neurotransmission (Langer, S.Z. and Arbilla, S., Trends in Pharmacol. Sci. 9 : 387, 1984).

In conclusion, in rat hippocampal slices, β-PEA inhibits the calcium-dependent evoked release of $^3$H-5HT through the activation of $\alpha_2$-adrenoceptors subsequent to the release of newly synthesized noradrenaline from a special pool of the transmitter.

243.6 A TONIC SEROTONERGIC INHIBITION ON NORADRENERGIC LOCUS COERULEUS CELL ACTIVITY AS DEMONSTRATED BY A SELECTIVE $5HT_1$ AGONIST : RU 24969. J.L. Brassard*, L. Quintin, G. Hilaire°*, C. Bardelay*, C. Oberlander* and J.F. Pujol* (SPON : R. Naquet ), Neuropharmacology, Roussel, 93230 Romainville and °CNRS LA 205, 13397 Marseille, France.

A serotonergic (5HT) input projecting to the noradrenergic (NA) locus coeruleus (LC) (Léger et al. C.R. Acad. Sci. Paris, 1980, 290 : 807-810) has been demonstrated to exert an inhibition on the activity of LC tyrosine hydroxylase (TH) (Renaud et al., Biochem. Pharmacol., 1975, 24 : 1739-1742). To assess whether this inhibition demonstrated on long term basis on the LC-TH would also be evidenced during short term studies, this 5HT/NA interaction was studied in the LC by electrophysiological, electrochemical and HPLC techniques. LC unitary activity (Hilaire et al., Soc. Neurosci. Abstr., 1984, 10, part I : p. 679), LC catechol oxidation peak recordings (Gonon et al., Brain Res., 1983, 273:207-216) and LC microdissections (Buda et al., Brain Res., 1983, 273 : 197-206) were performed as previously described. Combined electrophysiological and electrochemical recordings in the LC (Hilaire et al., 1984) evidenced an increase in unitary activity and catechol oxidation current after an injection of a selective $5HT_1$ agonist, RU 24969 (Hunt and Oberlander, "Serotonin", Plenum, 1981, 547-562) 1 $mg.kg^{-1}$ i.v. This increase in catechol oxidation peak was dose-dependent (ED50 = 1.4 $mg.kg^{-1}$ i.p. of RU 24969, log-probit method). HPLC determinations of DA and DOPAC on LC microdissections showed similar amplitude of variations as changes observed in catechol oxidation current. Furthermore, the 5HIAA/5HT ratio on the same LC microdissections was significantly depressed after RU24969 5 $mg.kg^{-1}$ i.p. Lastly, i.c.v. 5,7 DHT lesions (nomifensine pretreatment 10 $mg.kg^{-1}$ i.p.) almost totally suppressed the increase in catechol oxidation peak noticed after RU 24969 5 $mg.kg^{-1}$ i.p. The increased NA-LC activity after RU 24969 contrasts with the decreased 5HT turnover in the LC. It is consistent with the relief, by a selective 5HT1 agonist, of a tonic inhibition steming from 5HT neurons onto NA-LC perikarya. Such an interpretation is in keeping with the fact that iontophoretically applied 5HT on NA-LC cell suppress LC unitary activity (Segal, J. Physiol., 1979, 286 : 401-415). That 5,7DHT lesions suppress the increased LC cell activity suggests that 5HT1 receptors, stimulated by RU 24969, are located on 5HT neurons either at the level of the raphe perikarya or the 5HT terminals in the LC. This suggestion is reinforced by the fact that another 5HT1 agonist, PAT, iontophoretically applied on raphe dorsalis neurons suppresses their unitary activity (De Montigny et al., Neuropharmacology, 1984, 23 : 1511-1520).

243.7 LOCUS COERULEUS: AUTONOMIC INFLUENCE ON NEURONAL ACTIVITY AND BEHAVIOR. T.H. Svensson, M. Elam*, P. Thorén* and B. Persson*. Department of Pharmacology, Karolinska Institutet, Box 60400, 104 01 Stockholm, Sweden and Departments of Pharmacology & Physiology, University of Göteborg, Box 33031, 400 33 Göteborg, Sweden

In 1929 Cannon outlined various endogenous stimuli for sympathetic activation including haemorrhage, hypotension, hypercapnia, hypoxia and noxious stimuli. In a series of investigations we have showed that brain norepinephrine (NE) neurons in the LC respond with activation to the same stimuli. Here we report experiments demonstrating the cardiovascular and visceral influence on LC activity and evidence for behavioral consequences of such autonomic influence on brain function. Parallel recordings of single units in the LC and sympathetic splanchnic nerve activity (SNA) were performed in chloralhydrate anesthetized rats. Some behavioral results are also reported.

Both LC activity and SNA were highly responsive to peripherally induced alterations in blood pressure or blood volume. All responses of LC neurons were consistently abolished by bilateral, cervical vagotomy. In contrast, SNA changes in response to blood pressure variations were abolished by denervation of arterial baroreceptors but not by bilateral vagotomy. Generally, our results allow the conclusion that not only splanchnic nerves, but also LC neurons are regulated by cardiovascular afferents. However, whereas SNA is controlled by both arterial baroreceptors and cardiac volume receptors, the LC is controlled exclusively by cardiac volume receptors.

We have also found, that visceral events such as filling and emptying of the bladder and distal colon within a physiologically relevant range cause consistent and corresponding alterations in LC activity but not in SNA. Thus, the LC respond to a broad set of internal vegetative stimuli.

Peripheral, cardiovascular events such as changes in blood volume were associated with corresponding changes in synthesis and utilization of NE in brain regions innervated by the LC, i.e. cerebral cortex cerebellum, whereas other brain neurotransmitters such as serotonin (5-hydroxytryptamine, 5-HT) or dopamine (DA) did not show similar biochemically detected alterations. Interestingly, peripherally induced changes in blood volume were also associated with global changes in behavior. For example, volume load caused reduced exploratory activity in rats placed in a novel environment.

Consequently, endogenous autonomic stimuli may via the LC influence the response of the individual to external, environmental demands.

243.8 THE NUCLEUS LOCUS COERULEUS: EXTENSIVE EFFERENTS BUT RESTRICTED AFFERENTS AS REVEALED BY TRANSPORT OF DISCRETELY INJECTED PEROXIDASE-LABELED WHEAT GERM AGGLUTININ. G. Aston-Jones, M. Ennis, W. T. Nickell[1] and M. T. Shipley[1]. Dept. Biology, Wash. Sq. Cntr. Neural Sci., New York Univ., New York, NY 10003, and [1]Dept. Anat. Cell Biol., Univ. Cincinnati Col. Med., Cincinnati, OH 45267.

Knowledge of afferent cells that control discharge of locus coeruleus (LC) neurons is a critically necessary but lacking element in understanding the functions of this pervasive noradrenergic system. The sensitivity and specificity of transport techniques have increased substantially since previous investigations of LC afferents. Therefore, in this and a companion study (Ennis et al., this volume), we have initiated a comprehensive anatomic and physiologic examination of CNS systems that innervate LC.

In the present study, neurons afferent to LC, and efferent LC fiber trajectories, were investigated with discrete injections of wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP). Injections were made unilaterally into LC of chloral hydrate anesthetized rats using glass micropipettes (2-4 um tip diameter) filled with a 1% WGA-HRP solution. Survival times of 24-72 h were examined, and frozen 40 um-thick sections were reacted with tetramethylbenzidine. Data are reported here from a total of 4 animals with effective injection sites covering approximately 90% or more of the LC nucleus.

These injections consistently yielded a diverse and ubiquitous system of anterogradely labeled fibers. Pronounced fiber labeling was evident rostrally in, e.g., ipsilateral olfactory bulb and cerebrocortex and bilaterally in hippocampus, while intensely labeled fibers were found caudally in specific regions of the medulla and spinal cord. In particular, distinct patterns of lamination were apparent throughout ipsilateral cerebrocortex, and fiber pathways could be traced into the ipsilateral stria terminalis and external capsule. Labeled fibers were of moderate density in the central cervical nucleus of the spinal cord, bilaterally dense in the vestibular nuclei and inferior olive, and ipsilaterally very dense in the central nucleus of the amygdala.

In contrast to the extensive efferent fiber plexus, retrogradely labeled perikarya were quite restricted and generally few in number. Unlike previous reports, retrogradely labeled somata were not noted in the central amygdaloid nucleus, nucleus tractus solitarius or the spinal dorsal horn cell columns. The contralateral LC was notably devoid of labeled fibers or somata. Areas containing the most numerous retrogradely labeled neurons included the ipsilateral parabrachial and vestibular nuclei and the ventrolateral medullary reticular formation. Afferent innervation of LC also appeared to originate in the medullary parafasicular reticular formation and the cervical spinal gray matter.

Physiologic investigations of LC afferents in our companion study concur with these anatomic data. Supported by PHS grants NS 22320, AA 06607, and the Alzheimer's Disease & Related Disorders Assoc. to G. A.-J.

243.9 ELECTROPHYSIOLOGIC STUDIES OF NEURONS PROJECTING TO NUCLEUS LOCUS COERULEUS. M. Ennis, G. Aston-Jones and M. Segal[1]. Dept. Biology, Wash. Sq. Cntr. Neural Sci., New York Univ., NY, NY 10003, and [1]Weizmann Inst., Rehovot, Israel.

Previous anatomic reports indicate that locus coeruleus (LC) neurons receive afferents from diverse brain areas. Physiologic confirmation of these anatomic studies would substantiate their conclusions. Here, focal LC stimulation was used in an attempt to physiologically identify afferent neurons by antidromic activation. Putative afferent nuclei tested to date include ipsilateral rostral nucleus tractus solitarius (NTS; area of C2 adrenergic neurons), lateral reticular nucleus (LRN), and contralateral LC.

Adjacent paired microwires (50 um-diameter) were used for bipolar stimulation of LC in chloral hydrate anesthetized rats. Extracellular impulses were recorded from conventional glass micropipettes. Stimuli were 0.5 ms in duration, and 0.1-0.8 mA in amplitude. Driven cells were subjected to the following tests for antidromicity: (1) constant latency activation at threshold, (2) activation by pulse pairs of 5 ms intervals or shorter, and (3) collision between spontaneously occurring and driven impulses. All stimulation sites were histologically localized to LC, and recording sites were identified with dye deposited from pipette tips. Continuous low frequency stimulation (about 1/sec) was used to maximize detection of possible non-spontaneous cells during recording penetrations.

Of 9 neurons encountered in 11 histologically verified electrode penetrations through NTS, none were activated by stimulation of the LC. This result is consistent with our previous report (Ennis and Aston-Jones, Soc. Neurosci. Abstr., 1984) that lesions to this area do not alter discharge of LC neurons. This result is also supported by results of our companion study (Aston-Jones et al., this volume) that perikarya in NTS are not labeled following injections of retrograde tracer into LC.

Nine of 26 LRN cells recorded in 5 rats yielded constant latency and high frequency (up to 400 Hz) activation, but failed to exhibit collision between spontaneous and driven impulses. These neurons fired spontaneously at rates up to 20 Hz, and latencies for activation ranged from 1.5 to 3.0 ms. These results may reflect very secure synaptic driving from LC neurons, nearby structures, or fibers of passage. Two other neurons in this area were not spontaneously active, and were driven at latencies of 8 and 14 ms. These cells were shown to follow LC stimulation at frequencies over 300 Hz, but collision testing was not possible.

In 3 rats, 2 of 26 LC neurons recorded during contralateral LC stimulation were antidromically activated; however, the stimulation site for these driven cells was slightly ventral to LC. Eight other LC neurons were synaptically activated, but only at high stimulus intensities. Post-stimulus inhibition was found in all cells examined, with or without preceeding activation. The sparse antidromic activation observed here is consistent with results of our companion study (Aston-Jones et al., this volume) that retrograde labeling was not found between the loci coeruleus.

This work was supported by PHS grants NS 22320 and AA 06607.

243.10 PROJECTIONS OF PONTINE NORADRENERGIC CELLS TO CRANIAL NERVE NUCLEI: A RETROGRADE TRANSPORT AND IMMUNOCYTOCHEMICAL STUDY IN THE RAT. R. Grzanna and W.K. Chee*. The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

The origin of noradrenergic (NA) afferents to cranial nerve nuclei was studied by combining retrograde transport of True Blue (TB) with dopamine-ß-hydroxylase (DBH) immunocytochemistry. Discrete injections of TB were made into the motor nuclei of the trigeminal and facial nerve and into the sensory nucleus of the trigeminal nerve at rostral medullary levels (spinal trigeminal nucleus pars oralis) of rats. After 2-4 week survival, brainstem sections were processed for DBH immunocytochemistry using the indirect FITC method to visualize NA cells. Retrogradely labeled NA cells were counted in the locus coeruleus (LC), subcoeruleus (SC), and the A5-A7 cell group and their percentage with respect to the total number of NA cells within each groups was determined. Injections of TB into the trigeminal motor nucleus labeled primarily ipsilateral A7 cells (35%) and rostral A5 cells (18%). No labeled cells were seen in the LC and SC. Tracer injections into the facial motor nucleus labeled ipsilateral A5 cells (32%), contralateral A5 cells (13%) and A7 cells bilaterally (17%). Very few labeled cells ( 1%) were observed in the LC and SC. Injections of TB into the spinal trigeminal nucleus labeled cells in the LC and SC ipsilaterally (10 and 17%) and contralaterally (10 and 11%) in addition to a moderate number of cells in the A5 and A7 group. None of the injections labeled NA cells in the medulla.

A comparison of the labeling pattern of pontine NA cells after injections of TB into each of the 3 brainstem nuclei revealed striking differences: The NA innervation of the motor nucleus of the trigeminal nerve arises almost entirely from cells of the A7 group, while the NA innervation of the motor nucleus of the facial nerve arise primarily from the A5 group. The LC and SC do not project to these brainstem motor nuclei. In contrast, the NA innervation of the spinal trigeminal nucleus arises primarily from cells of the LC. The data indicate that NA afferents to motor and sensory nuclei of the brainstem arise from different subpopulations of NA neurons in the pons each of which has a restricted terminal distribution among brainstem motor and sensory nuclei. The NA innervation of the brainstem is not diffuse but is derived from separate NA cells projecting to restricted regions. This anatomic organization argues against a global influence of NA neurons on functionally diverse brainstem structures and suggests that subsets of NA cells may engage selectively restricted regions within the brainstem. (Supported by NS 16654, NS 21011 and NS 15199.)

243.11 PERSISTENCE OF NORADRENERGIC FIBERS IN THE CEREBELLAR CORTEX OF PURKINJE CELL DEGENERATION (pcd/pcd) MUTANT MICE FOLLOWING DEGENERATION OF PURKINJE CELLS AND GRANULE CELLS. B. Ghetti, S.Y. Felten, J.I. Nurnberger, M. Costanzo*, K. Perry*, R.W. Fuller, C.J. Alyea*, and D.L. Felten. Depts of Pathology and Psychiatry, Indiana University School of Medicine, Indianapolis, IN 46223, Dept. of Anatomy, University of Rochester School of Medicine, Rochester, NY 14642, and The Lilly Research Laboratories, Indianapolis, IN 46285.

Purkinje cell degeneration (pcd/pcd) mutant mice were examined during the course of Purkinje (P) cell death (26 and 35 day old) and at 3, 5, 9, and 12 months of age, by which time 90% of the granule cells also have degenerated (Ghetti et al, in preparation). The glyoxylic acid fluorescence histochemistry for catecholamines was used to investigate possible alterations or reorganization of the noradrenergic (NE) fibers from the coeruleo-cerebellar system in response to the degeneration of two major cell types in the cerebellar cortex, of which one, the P cell, is reported to be the major target neuron. In control mice, noradrenergic fibers traveled in linear and tortuous profiles through the granule cell layer, formed pericellular arrays alongside P cell somata, and branched profusely into both radially oriented (perpendicular to the surface) and longitudinally oriented chains. The density of NE varicosities diminished in the molecular layer with age. In pcd mutants, concomitant with the progressive shrinkage of the molecular layer, there was a progressive increase in the density of NE varicosities. This was most conspicuous at 9 and 12 months of age, at which time the molecular layer has been depleted not only of P cell dendrites, but also of parallel fibers. NE fibers in these zones formed dense parallel bundles of varicose profiles whose density reached 841.9% $\pm$ 178.6% (mean $\pm$ S.D., n=3) at 12 months of age, compared with age-matched controls. Neurochemical measurement of NE content in whole cerebellum of the pcd mutants revealed no change compared with age-matched controls. We conclude that noradrenergic innervation persists in the cerebellar cortex despite the death of P cells and most of the granule cells. Although we found an increased density of varicosities in the molecular layer of mutant mice, progressing with age, we believe that this can be explained on the basis of the resultant geometry of the altered cerebellar cortex. It appears that the health of the environment surrounding the NE fibers in cerebellar cortex has little influence on their anatomical integrity. Supported in part by NIH grant NS 14226 to B.G. and by a MacArthur Foundation Prize Fellowship (DLF).

243.12 MORPHOLOGICAL AND HISTOCHEMICAL CHARACTERISTICS OF DOPAMINE-CONTAINING NEURONS OF THE MAMMALIAN SPINAL CORD, IN VITRO AND IN VIVO. A.P. Mariani*, M.T. Caserta and J.L. Barker. Laboratory of Neurophysiology, NINCDS, NIH, Bethesda, MD 20205

Although catecholamine-containing neurons of the brain are thought to project to the spinal cord, no cell bodies of intrinsic spinal cord neurons are known to be catecholaminergic. Now, by using an aldehyde method for the fluorescent visualization of catecholamines, and immunohistochemistry, the morphology of three distinct classes of dopamine-containing neurons from the spinal cord have been revealed in vitro and in vivo. Dissociated cultures of spinal cords from embryonic (E) day 13 mice or E 14 rats, devoid of dorsal root ganglia, were maintained according to well-established protocols. Five percent of the cultured neurons fixed by the formaldehyde-glutaraldehyde (Faglu) method displayed the bluish-green fluorescence characteristic of catecholamines, and were of three distinct morphologies. The most common type of catecholamine-containing spinal cord neuron was a small bipolar cell (63 percent), with a 16-20 µm diameter cell body and two thick processes projecting from opposite poles of the cell. The second type of catecholamine-containing neuron (27 percent) was relatively large with a 50 µm diameter flattened perikaryon which gave rise to a variable number of varicose and/or spiny processes. Medium-sized multipolar cells with 25-30 µm diameter cell bodies constituted the third type of catecholamine-containing neuron (10 percent) and had four to six processes with secondary and tertiary branches radiating outward from the perikaryon. To better ascertain the identity of the catecholamine, cultures were also stained with antibodies to the catecholamine synthesizing enzymes, tyrosine hydroxylase (TH), dopamine-ß-hydroxylase (DßH) and phenylethanolamine-N-methyltransferase (PNMT). Cells identical in morphology to the types detected by Faglu fluorescence were immunoreactive for TH, but not DßH or PNMT. To find if there were in vivo correlates to the in vitro results, we treated adult rats with a monoamine oxidase inhibitor and perfused them with Faglu. Sections of the thoracic spinal cord displayed catecholamine-containing neurons similar in size and morphology to the small bipolar cells in culture. These neurons had a rostral-caudal, V-shaped organization, beginning in the gray matter near the central canal and extending into the white matter adjacent to the intermediolateral cell column.

244.1 A GRADED RETROGRADE MEMORY IMPAIRMENT AFTER LESIONS OF THE BASAL FOREBRAIN IN THE RHESUS MONKEY.
M.B. Moss and D.L. Rosene. Department of Anatomy, Boston Univ. Sch. of Med., Boston, MA, 02118.

Over the past few years, evidence from neuropathological studies has implicated structures in the basal forebrain (nucleus basalis of Meynert and substantia innominata) in the severe memory impairment of Alzheimer's disease (as well as of other dementia and amnesic disorders). More recently in animals, direct evidence of basal forebrain involvement in memory function has begun to emerge. We now report our observation of a graded retrograde memory impairment following basal forebrain lesions in the rhesus monkey. Seven normal adult rhesus monkeys were trained preoperatively for one and one-half years on a battery of discrimination learning and visual memory tasks. Over the course of this preoperative phase, a total of five different two-choice pattern discrimination problems were administered, at approximately three month intervals, within a series of recognition and associative memory tasks. For the animals designated as the basal forebrain group, the last discrimination problem was administered two days before surgery. Upon completion of this phase of testing, four of the animals received two-stage bilateral ablations of the basal forebrain under direct visual guidance by intracerebral injection of the neurotoxin, ibotenic acid. The remaining three animals served as an unoperated control group. Following three weeks recovery from surgery and one week of other behavioral testing, all animals were retested on the five preoperatively acquired pattern discrimination problems. Monkeys were retrained on one discrimination problem per day and the pairs of patterns were presented in the same order that they were administered preoperatively. For the normal control animals, savings scores for the first pattern discrimination were 82, 100 and 100 (mean score of 94). The four monkeys with BF lesions performed equally well, obtaining saving scores of 83, 88, 96 and 100 (mean score of 92). On the subsequent four pattern discrimination problems, normal monkeys achieved group mean savings scores of 96, 92, 91 and 93 respectively. In contrast, the BF group showed significantly diminished savings over the four pattern discriminations with means of 61, 68, 52 and 23, respectively. The results not only reveal a retrograde memory impairment for associative learning as a consequence of basal forebrain lesions, but also point to a temporal gradient, characterized by a greater savings of remote, as opposed to recent, preoperative learning. Further, these findings suggest the possibility that patients in the early stages of Alzheimer's disease who demonstrate a temporally graded retrograde impairment, may reflect those cases in which neuropathological changes begin in, or primarily affect, the basal forebrain.

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244.2 PATTERNS OF ACHE LOSS IN THE HIPPOCAMPAL FORMATION OF MONKEYS FOLLOWING LESIONS OF THE BASAL FOREBRAIN: A QUANTITATIVE HISTOCHEMICAL STUDY. D.L. Rosene and M.B. Moss. (SPON: B. Payne) Dept of Anatomy, Boston University School of Medicine, Boston, MA, 02118.

The basal forebrain of the monkey contains a large population of cholinergic neurons which are known to project to the cerebral cortex and limbic system. However, the specific pattern and extent of cholinergic loss within target sites after basal forebrain damage is not known. Using the enzyme acetylcholinesterase (AChE) as a marker for cholinergic activity, we have employed a quantitative histochemical approach to assess the extent and pattern of cholinergic depletion in the hippocampal formation in monkeys following restricted lesions within the basal forebrain. Under direct surgical exposure, three rhesus monkeys received bilateral lesions of the basal forebrain by intracerebral injection of the neurotoxin, ibotenic acid. Following extensive behavioral testing, the monkeys were sacrificed and then perfused transcardially with quarter-strength Karnovsky's fixative. The tissue was then processed with both the Karnovsky-Roots and Koelle procedures for identification of AChE. The reaction product of the Karnovsky-Roots procedure, copper ferrocyanide, was quantified using a scanning and integrating microdensitometer set at an absorbtion spectra of 485 nm. On the basis of other studies in this laboratory which demonstrate basal forebrain efferent termination in the hippocampal formation, measurements of AChE were made within all laminae of hippocampal sectors CA3, CA1 and the dentate gyrus, as well as in selected sites in the subicular complex. The measurements were taken at four equally spaced rostro-caudal levels throughout the length of the hippocampal formation. In order to control for interslide variability, all measures were expressed as a percentage of the AChE density in the tail of the caudate measured in the same section. Extensive loss of AChE was, on the whole, limited to the most rostral (uncal) part of the hippocampal formation. Within the uncal hippocampus, loss of AChE occured in CA1, the adjacent prosubiculum, specific laminae of CA3, and the presubiculum. In the three more caudal levels of the hippocampal formation, loss of AChE density was found only within specific areas of the subicular complex. The relationship between damage in specific areas of the basal forebrain and the resulting loss of cholinergic activity in the hippocampal formation may help in our eventual understanding of how these two regions may interact in the neuropathological basis of the characteristic memory disorder of Alzheimer's Disease.

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244.3 MEMORY DEFICITS IN MONKEYS WITH MEDIODORSAL THALAMIC LESIONS.
E.C. Gower, D.L. Kasdon*, S. Jacobson and N.M. Butters. V A Medical Center, Boston, MA 02130.

The mediodorsal nucleus of the thalamus (MD) is frequently involved in the diencephalic pathology accompanying human memory disturbances. The present ongoing experiments explore the contribution of MD to mnemonic processes by examining (1). the retention of preoperative discrimination learning and (2). the acquisition and retention of new material in monkeys (M. fascicularis) with medial thalamic lesions.

Electrolytic lesions were made bilaterally in the mediodorsal nucleus following retraction of the walls of the interhemispheric fissure, section of the splenium of the corpus callosum, and visualization of the thalamus. The position of MD was calculated and the lesion electrode placed stereotaxically using the observed location of the habenular commissure as coordinate zero. Controls include monkeys subject to splenium section, and normal animals. The lesions have not yet been histologically confirmed.

After a postsurgical recess, monkeys were tested for the retention of three 2-choice visual pattern discriminations which had been learned individually six, three and one week prior to surgery. The three pattern pairs were relearned postoperatively as a concurrent discrimination problem. The experimental group displayed a modest overall deficit in relearning compared with the high level of savings shown by normal and operated control groups. No systematic difference appeared in the retention of individual pattern pairs for normal and control monkeys. In contrast, monkeys with thalamic lesions exhibited a significantly lower degree of retention for the immediate presurgical problem only ($t(4)=2.41$, $p<.05$ (one-sided test)).

All monkeys were further tested with a trial-unique, non-matching-to-sample test of object recognition memory, with sample objects drawn from a pool of 700. Compared with normals and controls, experimental monkeys were moderately impaired in learning the non-matching problem with a delay of 10 seconds, and were further impaired in performance on longer delays. These data indicate that medial thalamic lesions may retard the acquisition of a new rule governing discrimination behavior as well as impair retention of trial-specific events. Moreover the relearning deficit which is specific to pattern pairs presented shortly prior to surgery is reminiscent of the temporally graded retrograde memory defect of amnestic patients. (Supported by VA Medical Research funds).

244.4 SPARING OF VISUAL RECOGNITION AFTER NEONATAL LESIONS OF INFERIOR TEMPORAL CORTEX IN INFANT RHESUS MONKEYS.
C. Hagger*, M. Brickson* and J. Bachevalier. Lab. of Neuropsychology, NIMH, Bethesda, MD 20205.

Visual recognition measured by preferential viewing develops early in infancy and, like visual recognition measured with problem-solving tasks in adults, is dependent on the integrity of limbic structures (Brickson and Bachevalier, Soc. Neurosc. Abstr., 10:137, 1984). Since visual recognition measured with problem-solving tasks in adults is also dependent on inferior temporal cortex (area TE) (Mishkin, Phil. Trans. R. Soc. Lond., B 298:85, 1982), we tested whether removal of this cortical area in infancy would, like neonatal limbic lesions, impair visual recognition measured by preferential viewing.

Twelve infant and 8 adult rhesus monkeys served as unoperated controls, and 3 infants and 3 adults received bilateral area TE lesions. The infants received their bilateral ablations in two stages, at 7 and 21 days of age, while the adults received theirs in one stage. Separate sets of trial-unique objects were shown to the infants at 5, 15, and 30 days of age, while only the first set was presented to the adults. For each of ten trials, animals were exposed to a pair of identical objects for a 30-sec familiarization period, and then, after 10 seconds, the previously exposed object was paired with a new one for two 5-sec recognition periods, with the position of the 2 objects being reversed from one period to the next. Percent of fixation time (PFT) spent on the novel object was measured for each trial.

Like normal adults, the normal infant monkeys between 15 and 30 days of age gazed at the novel stimuli more than at the stimuli already gazed at several seconds earlier (mean PFT: 54%, 66%, and 73% for 15 days, 30 days, and adults, respectively). Surprisingly, however, even though the visual preference for novelty was completely absent in adults with bilateral area TE lesions (mean PFT: 49%), it was preserved in both the 15-day-old infants with unilateral area TE lesions and the 30-day-old infants with bilateral area TE lesions (mean PFT: 64% and 66%, respectively).

These findings support earlier data showing that visual recognition approaches adult levels even in early infancy and suggest further that, although area TE is involved in recognition memory in adults, neonatal removal of this cortical area leads to significant sparing of this function.

244.5 EVIDENCE FOR INVOLVEMENT OF PREFRONTAL CORTEX IN COGNITIVE CHANGES DURING THE FIRST YEAR OF LIFE: COMPARISON OF PERFORMANCE OF HUMAN INFANTS AND RHESUS MONKEYS ON A DETOUR TASK WITH TRANSPARENT BARRIER. A. Diamond and P. Goldman-Rakic, Section of Neuroanatomy, Yale School of Medicine, New Haven, CT 06510.

Infants of 7½-12 months show a developmental progression in performance of Piaget's classic AB task, which taps the child's ability to remember the location of a hidden object. We have shown earlier that lesions of prefrontal cortex in adult monkeys selectively impair AB performance, reducing it to the level of 7½-9 month old human infants (Diamond & Goldman-Rakic, Neurosci. Absts., 1983). We now report that prefrontal monkeys perform like 7½-9 month old human infants on Object Retrieval (OR), a detour problem requiring the subject to suppress the tendency to reach directly to an object. These findings are relevant to the neural basis of cognitive development.

Ss were 25 human infants, tested biweekly from 6-12 months, and 9 adult rhesus monkeys (Macaca Mulatta): 3 with bilateral lesions of dorsolatal prefrontal cortex, 3 with bilateral lesions of parietal cortex, and 3 unoperated.

On each OR trial, a bait (toy for infants, food for monkeys) was placed inside a transparent box open on one side. Experimental variables included: (a) side of box opening (left, right, front), (b) position of box (far left, far right, middle), and (c) position of bait in box (partially out, inside near opening, deep in box). A fully crossed experimental design was used with order of conditions counterbalanced across testing sessions. The bait was always visible, but the experimental variables jointly controlled whether bait was seen through the open or closed sides of the box.

Infants of 7½-9 months and prefrontal monkeys needed to have seen bait through the opening in order to reach in and retrieve it. Otherwise, they reached only at the closed sides of the box through which they saw bait. They also displayed an "awkward reach" when the left or right sides were open, i.e., they reached with the hand farthest from the opening. In contrast, 12 months old infants and intact, and parietal, adult monkeys reached into the box without having looked in, and used the hand nearest the opening.

These results demonstrate a link between the prefrontal cortex and a task quite different from AB. Object Retrieval is not a hiding task, nor is AB a detour task. However, both tasks require association of events separated in time (AB) or space (OR) and both require inhibition of prepotent responses (in AB: tendency to repeat previously rewarded response; in OR: reaching directly to goal). These cardinal functions, impaired by frontal lobe lesions in both monkeys and man, develop during the first year and establish the basic structure for future cognitive achievements.
Supported by MH-38546, MH-00298, Fellowship MH09007, NSF BNS-8013-447, and the Sloan Foundation.

244.6 A COMPARISON OF CORTICAL AND SUBCORTICAL MEMORY IMPAIRMENTS M. El-Awar* , J.T. Becker, K.M. Hammond* , & F. Boller* , Depts. of Neurology & Psychiatry, Univ. Pittsburgh, Pittsburgh, PA 15213

Alzheimer's Disease (AD) in its early stages affects primarily areas of neocortex, and is often considered a cortical dementia. Parkinson's Disease (PD), on the other hand, primarily affects subcortical areas, and the dementia associated with PD differs from that of AD. In order to compare the nature and extent of the memory loss in these two dementia syndromes, patients with AD (n=12), PD (n=12), and normal control subjects (n=12) were given a battery of neuropsychological tests which included a detailed study of their verbal learning abilities.

All of the subjects were given a short battery of tests to assess the extent of their cognitive decline, including tests of verbal fluency and confrontation naming. The verbal paired-associate learning test consisted of teaching the subjects ten arbitrary associations between word pairs. Training continued until they had either learned all 10 pairs, or they had been tested for 10 trials. One hour after the completion of the acquisition training, the patients were given two tests of recall and two tests of recognition memory to examine their ability to remember the response words, and the specific associations between stimulus and response words.

The patients with AD and PD learned the paired-associates at a slower rate than the controls. The AD patients, however, learned significantly less than the PD patients, in part due to the greater severity of the dementia in AD. Performance of the delayed memory tests clearly distinguished the groups of patients. The PD patients recalled less than the control subjects, while the AD patients, who learned few associations, recalled essentially none. On the recognition tasks both the controls and the PD patients were equally proficient at recognizing the response words and the correct associations. However, the AD patients, although relatively good at recognizing the response words, were unable to recognize the correct associations. Thus, although they had stored information about the response words, they had not stored information about the specific associations.

There were also differences in the types of errors made by the patients during learning. When the AD patients were incorrect, they tended to make semantic associations to the stimulus words (e.g., honey-bee for honey-thread). In contrast, the patients with PD made errors by responding with semantic associations to the response words (e.g., honey-string).

These data, therefore, suggest that the patterns and types of memory impairment in the dementias of AD and PD are different. These differences do not appear to be related to the relative severity of the overall cognitive impairment and may reflect the differential contribution of the CNS structures damaged in AD and PD to memory processing.

244.7 IMPAIRED SPATIAL MAPPING AFTER DISRUPTION OF SOME ROSTRAL CONNECTIONS OF THE HIPPOCAMPAL SYSTEM IN RATS. R. J. Sutherland, G. Prusky*, R. H. Dyck and A. Rodriguez*, Dept. of Psychol., The Univ. of Lethbridge, Lethbridge, Alberta, Canada T1K 3M4.

The integrity of the hippocampal formation (HPC) and retroHPC area is essential for normal use of mapping strategies in solving spatial problems. This has been most clearly demonstrated in rats using the place navigation task (Morris, R. et al., Learn. & Motiv., 12:239, 1980; Nature, 297:681, 1982; Sutherland et al., Soc. Neurosci. Abstr., 6:565, 1980; Behav. Brain Res., 7:133, 1983). Damage to intrinsic HPC components impairs performance in situations requiring either acquisition or retention of spatial mapping (Sutherland et al., Soc. Neurosci. Abstr., 9:638, 1983). In the following four experiments we examine the contribution of HPC connections in the fornix/fimbria to acquisition and usage of mapping and non-mapping spatial localization strategies.

In the first experiment normal rats and rats with fornix/fimbria transection were trained to navigate to an invisible, fixed platform and to a visible, fixed platform in a large circular pool. Unlike normal rats, the fornix/fimbria transected rats could not learn to swim directly to the invisible platform, instead they learned to solve the problem using a stereotyped circumnavigation strategy. Both groups swam directly to the visible platform. Rats with additional bilateral damage to the overlying cingulate cortex were impaired at locating the visible platform and did not acquire an efficient non-mapping strategy to locate the invisible platform. In the second experiment rats were implanted with a stimulation electrode in the fornix and bilateral EEG electrodes in the dorsal HPC and posterior neocortex. All rats acquired a mapping strategy to locate the invisible, fixed platform. Place navigation was disrupted for 60 - 300 sec following fornix stimulation (40 μA, 100 pps, .2 ms for 15 sec) even though HPC and neocortical EEG was unaffected at these intervals. Stimulation immediately after learning a new location disrupted navigation at much longer post-stimulation intervals.

Microinjections of the retrograde fluorescent dye True Blue into the basal forebrain confirmed the existence of major projections to the nucleus accumbens from several limbic structures, most importantly from ipsilateral subiculum, CA1, amygdala, entorhinal cortex, perirhinal cortex, and olfactory bulbs. These structures project differentially to medial and lateral nucleus accumbens. Electrolytic lesions were placed in medial lateral or both medial and lateral nucleus accumbens and the rats were subsequently trained to navigate to the fixed, invisible and fixed, visible platforms. Navigation to the invisible platform was impaired.

These results emphasize the importance of some rostral HPC system connections, particularly with both the acquisition and usage of spatial mapping strategies.

244.8 THE ONTOGENY OF MAPPING AND NON-MAPPING SPATIAL STRATEGIES FOLLOWING NEONATAL HIPPOCAMPAL DAMAGE IN RATS. R. H. Dyck, R. J. Sutherland and M. R. Buday*. Dept. of Psychol., The University of Lethbridge, Lethbridge, Alberta, Canada T1K 3M4.

The ability to use spatial mapping strategies is a late-developing cognitive skill in rats and humans. In the place navigation task, rats do not use an adult-like mapping strategy until some time after the 4th week of life, by which time the hippocampal formation (HPC) has attained a nearly adult degree of anatomical and physiological maturity (Schenk, F., Behav. Neural. Biol., 43:69, 1984). In the present experiments the ontogeny of mapping and non-mapping spatial strategies was examined in normal rats and rats with HPC and/or posterior neocortical damage sustained in infancy.

Within 48 hr after birth rat pups were anesthetized by hypothermia and vinblastine, vincristine or colchicine (.06 - .3 μg/.5 μl) was microinjected bilaterally into HPC or posterior neocortex. Beginning on the day of eye-opening (approx. day 15) the rats received 4 trials daily in the place navigation task with a fixed, invisible platform or a fixed, visible platform. By day 19 all rats were able to swim almost directly from any starting location to the visible platform. Prior to day 24 of life the behaviour of the normal, HPC- and posterior neocortex-damaged rats was indistinguishable in the invisible platform condition. After day 24 of life the control rats began to swim almost directly to the invisible platform from any starting location; the rats with extensive HPC or posterior neocortex damage were never able to navigate directly to the invisible platform. When re-tested as adults the HPC-damaged rats demonstrated acquisition of an efficient non-mapping strategy, involving stereotyped circumnavigation of the pool. Rats with incomplete or unilateral damage eventually learned to navigate directly to the invisible platform.

The results are consistent with the idea that the post-natal maturation of the HPC selectively underlies the ontogeny of cognitive mapping ability and that neonatal HPC damage is associated with little or no sparing of mapping functions.

244.9 DIFFERENTIAL CONTRIBUTION OF PARIETAL CORTEX AND HIPPOCAMPAL FORMATION IN THE RETENTION OF A COGNITIVE MAPPING TASK. B. V. DiMattia* and R. P. Kesner, Dept. of Psychology, Univ. of Utah, Salt Lake City, Utah 84112.

The hippocampal formation (HF) has been labeled the locus of the 'cognitive map' in the brain (O'Keefe & Nadel, 1978). Many studies have demonstrated HF involvement in the acquisition of cognitive mapping tasks, although there is conflicting evidence regarding the role of HF in the retention of such tasks.

However, based on symptomatology from brain damaged humans, one might be tempted to argue that the parietal cortex (PC) is more important for spatial information processing than HF. Parietal lobe patients are impaired in both the acquisition and retention of spatial or geographic information.

In a previous study both PC and HF damaged rats were impaired in the acquisition of the Morris water maze task (DiMattia & Kesner, 1984), which is considered to require cognitive mapping (Morris et al., 1981). The present experiment tested the effects of PC and HF lesions on the retention of this task. Rats were given varying degrees of preoperative training (40 to 80 trials) prior to receiving complete PC (aspiration) or HF (electrolytic) lesions.

Following a 7-10 day recovery period the animals were given 40 retention trials in the maze. The results indicated that, in terms of latency to find the hidden platform, the PC animals were impaired across all postoperative trials, regardless of the number of preoperative trials. However, in general, the HF animals were less impaired than the PC animals and improved across postoperative trials such that their latencies were within normal limits for the last 20 trials in both the 40 and 80 preoperative trial conditions. However, both HF and PC damaged rats were impaired relative to controls in their directional heading toward the hidden platform, regardless of whether the animals had 40 or 80 preoperative trials. This dissociation in performance measures might be accounted for by the fact that a large percentage of the HF lesioned animals tended to use a 'praxic' strategy to find the hidden platform (i.e., to swim in a concentric pattern until they encountered the platform), although some PC rats also employed such a strategy.

These data strongly implicated a role for PC in the retention of spatial information in the rat in tasks that require cognitive mapping. It cannot, however, be argued conclusively that the HF does not play a role in the retention of the task, since there was no recovery for directional heading toward the platform.

244.10 DORSOMEDIAL THALAMIC LESIONS: POST-OPERATIVE IMPAIRMENT OF RADIAL MAZE PERFORMANCE, BRIGHTNESS DISCRIMINATION LEARNING AND CHANGES IN OPEN FIELD ACTIVITY. K. A. Stokes* and P. J. Best. Dept. of Psychology, University of Virginia, Charlottesville, VA, 22901. (SPON.: J. Hahn)

The role of the dorsomedial thalamus (DMT) in memory is not yet clearly understood. Through its connections to frontal cortical and hippocampal systems, a role in spatial information processing might be postulated. Yet results here are equivocal. DMT lesions have variously been found to have no effect on post-operative acquisition of a radial maze habit (Kolb, Sutherland and Whishaw, Behav. Br. Res., 6, 1982, 365) and to produce marked impairments on spatial task performance (Kessler, Markowitsch and Otto, J. Comp. Physiol. Psychol. 96, 1982, 712; B. McNaughton, personal communication). The present experiment investigated behavioural consequences of DMT lesions on three tasks: a pre-operatively well-learned delayed memory for 4 choices task on the 8-arm radial maze; brightness discrimination on a T-maze, and open field activity.

Thirteen male Long-Evans rats were pre-operatively overtrained on the 8-arm maze habit, followed by the delayed memory for 4 choices task. This involved forcing the animals to respond to 4 arms of the maze (the daily sequence of which varied in a random manner), then, after a one hour delay in which the animals were removed from the maze, requiring them to respond to the 4 previously-blocked arms for reinforcement. Radial maze testing, as well as all other behavioural testing,was conducted in a dimly-lit, visually-deprived environment. DMT lesions were performed on half of the subjects after the task had been well-learned (approx. 80 trials). Four days later, testing was resumed. Post-operative tesing revealled that DMT lesioned animals were severely and lastingly impaired on the post-delay 4 choices. Number of entries into incorrect arms, as well as number of perseverative errors increased significantly in the lesioned compared to sham operated animals. Pre-delay responding was impaired as well. Subsequent testing on the simple 8-arm maze task also yielded significantly poorer performance by DMT rats, due mainly to the development of rigid, maladaptive response strategies. Neither could the DMT rats acquire a brightness discrimination task in a T-maze: they instead developed position habits and perseverated on these, regardless of reinforcement contingency. On the third task, open field testing, DMT animals exhibited greater activity levels and enhanced thigmotaxis compared to sham controls.

Thus, DMT animals are impaired on spatial tasks, especially in an environment where minimal cues are available to guide responding. Also, DMT animals exhibit hyperactivity and perseveration which compete with adequate solution of the task at hand.

244.11 LESIONS OF THE NUCLEUS BASALIS MAGNOCELLULARIS IMPAIR MAZE LEARNING IN RATS. D.J. Hughey, G.W. Wenk, and D.S. Olton, Dept. of Psychology, The Johns Hopkins University, Baltimore, MD 21218

Damage to the basal forebrain cholinergic system has been associated with learning and memory impairments in both humans and animals (Bartus et al., 1982; Hepler et al., 1985). The following experiment examined the maze learning ability of rats with lesions of one component of this system, the nucleus basalis magnocellularis (NBM). Bilateral lesions were made in male, Sprague Dawley rats by the microinfusion of ibotenic acid into the NBM, which decreased the level of cholinergic markers in frontal cortex by approximately 45%. Control operations consisted of infusions of phosphate-buffered saline.

Rats were food-deprived to 85% of their ad lib. weights and began maze training within 7-10 days of surgery. The maze was a 14 unit T maze whose walls were constructed of 18" high Plexiglas. A curtain surrounded the perimeter of the maze in order to lessen the availability of extramaze cues. Each rat was given one trial daily for thirty days. An error was recorded each time a rat entered a blind alley.

At the beginning of training, both groups averaged approximately 15 errors per trial. The performance of both lesioned and control rats improved with training, but the latter did so at a faster rate. By the end of training, control rats had an average error score of approximately 2 errors per trial. In contrast, the lesioned rats finished training with an average of approximately 7 errors per trial.

These results demonstrate that the functional integrity of the NBM and its projection areas is important for learning the correct path through a maze. Thus, these data add to our understanding of the association between central cholinergic activity and behavior, and they may have implications for the study of animal models of the cholinergic component of Alzheimer's Disease.

244.12 Cholinergic Neurons, Learning and Recovery of Function. L.E. Harrell, T.S. Barlow* and D. Parsons*. Department of Neurology, V.A. Medical Center and University of Alabama, Birmingham, AL 35294.

Central cholinergic activity has been implicated in learning/memory processes as well as spatially cued behavior. The following two experiments were designed to evaluate the role of the cholinergic septohippocampal projection (SHP) in these types of behaviors. Both experiments employed as a behavior task the radial 8-arm maze (RAM) since performance of this task appears to be dependent on both cholinergic activity and hippocampal integrity.

Exp. I examined the interaction of individual learning strategies and recovery of function following destruction of the SHP. Ten adult rats were trained to approach 8 baited arms of a RAM. Five rats appeared to acquire the task by choosing arms non-sequentially, relying on distal environmental cues (spatial strategy; SS), while 5 employed sequential arm entry (non-spatial strategy; NSS). Rate of initial task learning was not different between groups. Lesions of the SHP result in disruption of task performance in both groups preoperatively. Postoperatively, animals employing a SS preoperatively switched to a NNS, and relearned the task significantly faster than those employing a NSS preoperatively, who continued to use this strategy.

Results of Exp. I suggested that the cholinergic SHP was important for the maintenance of spatially mediated behavior. Exp. II addressed this issue by examining acquisition of a modified version of the RAM (where only 4 arms are baited, thereby blocking the use of NSS), after SHP destruction. Control animals easily mastered the task in 39.7 $\pm$ 5.0 trials. Learning was observed over time with reduction of total selections, errors, and responses to baited and unbaited arms. Lesioned animals showed no evidence of learning after extended trials. They did develop over time some response sequencing strategies so that arm choices were no longer random.

The data from these two experiments suggest that (1) the cholinergic SHP is important for the acquisition and maintenance of spatially mediated behaviors, (2) different learning strategies can be employed to acquire the same behavior, (3) rate of recovery after brain injury can be influenced by preoperative learning strategies, and (4) loss of cholinergic function can modify learning strategies. These results suggest that losses of cholinergic neurons in Alzheimer's Disease may lead to different deficits depending on pre-morbid learning strategies.

245.1 EFFECTS OF INTRASEPTAL INFUSION OF GABA AND L-GLUTAMATE ON THE RAT'S ACOUSTIC STARTLE RESPONSE. O. A. Elabanjo* and J. H. Evans*. (Spon: R.Neuman) Department of Psychology, Memorial University of Newfoundland, St. John's, Newfoundland, Canada A1B 3X9

It has been shown that lesioning the medial septum (MS) results in increased amplitude of the rat's acoustic startle response (ASR) [Miller and Treft. Physiol. and Behav., 23, 645, 1979], but because lesioning destroys cell bodies, and terminals as well as fibers coursing through the MS, this type of treatment does not permit further analysis as to which aspect of the MS is involved in startle modulation.

To this end, the present study set out to use cell-specific inactivation by infusing 1M GABA, and cell-specific excitation by infusing 0.5M L-Glutamate into the MS. 0.9% w/v saline was used as the control vehicle. Thirty male Sprague-Dawley rats, divided into three equal groups were infused with 1.5ul of one of these solutions immediately before being presented with 80 trials of 110db white noise at a 10 sec. inter-trial interval. The premise was that GABA should act to increase the ASR's amplitude on early trials, saline should have no effect, while L-Glutamate should decrease it. Since GABA and L-Glutamate have been shown to act on cell bodies, any effect of the treatment may then be attributed to the actions on the cell bodies in the MS and not the fibers.

The results show that L-Glutamate significantly depressed startle on the first 5-trial block relative to the saline control. GABA-infused rats did not differ from saline control on this measure. Cannula placements were verified by histological methods in all subjects.

245.2 LONG-TERM POTENTIATION AND THE SUSTAINED INCREASE IN GLUTAMATE RELEASE WHICH FOLLOW TETANIC STIMULATION OF THE PERFORANT PATH ARE BOTH BLOCKED BY D(-)AMINOPHOSPHONOVALERIC ACID. M.A. Lynch, M.L. Errington and T.V.P. Bliss (SPON: W.C. Abraham). National Institute for Medical Research, Mill Hill, London NW7 1AA, U.K.

D(-)aminophosphonovaleric acid (APV), a specific antagonist of the NMDA subtype of glutamate receptor, blocks the induction of long-term potentiation (LTP) in the CA1 region of the hippocampus without interfering with synaptic transmission, as first described by Collingridge et al., (J. Physiol, 334, 33, 1983). We have found that D(-)APV has a similar blocking effect on LTP in the dentate gyrus. Using a push-pull cannula with attached electrodes we have examined the relation between the effect of the drug on LTP and its effect on glutamate release. Rats anaesthetized with urethane were perfused with artificial csf for 30 min, and then with csf containing 100 uM D(-)APV for 35 min. Perfusion with control csf was then continued for a further two hours. A control group was perfused with artificial csf alone for three hours. (D)-APV did not significantly affect release, and only slightly reduced the slope of the epsp; the population spike however was reduced by an average of 54% after 35 min. A tetanus (250 Hz for 200 msec) during perfusion with APV failed to produce either LTP or the sustained increase in release of endogenous glutamate which we have previously shown to accompany LTP (Bliss et al., J.Physiol, 361, 50P, 1985). A second tetanus, given after APV had been washed out, induced both LTP and an increase in release of endogenous glutamate (see Table 1). In a separate series of experiments, D(-)APV was found to depress the $K^+$-induced, $Ca^{2+}$-dependent release of preloaded $^{14}C$ glutamate from dentate slices, implying the existence of presynaptic receptors for APV. The possibility exists therefore that the blocking of LTP by APV may be mediated by a presynaptic mechanism.

| | 1st hr | 2nd hr | 3rd hr | 1st hr | 2nd hr | 3rd hr |
|---|---|---|---|---|---|---|
| | glutamate release | | | epsp slope | | |
| APV (n=8) | 100 (14) | 64 (12) | 175 (27) | 94 | 99 | 120 |
| control(n=8) | 100 (12) | 47 (5) | 67 (9) | 99 | 93 | 92 |

Table 1. Values for glutamate release are group averages of one hour means (±SEM), expressed as percentages of mean values in the first hour. Values for the slope of the epsp are 10 min means obtained at the end of each hour, expressed as percentages of mean values obtained during the first 30 min. Test shocks were given every 30 seconds, and tetani were delivered at 60 and 120 min.

245.3 LONG-TERM ASSOCIATIVE POTENTIATION/DEPRESSION AS AN ANALOGUE OF CLASSICAL CONDITIONING. B.Burger and W.B Levy. Dept. of Neurosurgery, Univ. of Virginia Sch. of Med., Charlottesville, VA 22908.

Associative long-term potentiation as studied at the entorhinal cortex-dentate gyrus (EC-DG) synapses may be considered an analogue and candidate process for the actual synaptic changes subserving classical conditioning (CC). In this analogy the response (UCR) is the synaptic response evoked by the ipsilateral EC input (analogue of the UCS). Stimulation of the converging contralateral EC afferents provides the analogue to the CS. If the baseline contralateral response is arbitrarily called subthreshold, then associative activation of both ipsi- and contralateral inputs produces a potentiated response above threshold which resembles the ipsilateral response. Conditioning the contralateral afferents alone does not produce this increase. Furthering this analogy are the effects of preacquisition UCS exposure and preaquisition backward conditioning. Both preexposures depress acquisition of the classically conditioned nictitating membrane response, with backward conditioning more effectively retarding acquistion than simple UCS preexposure[1]. Similarly, using standard procedures for studying contralateral EC-DG synaptic modification[2,3], backward conditioning (ipsilateral then contralateral conditioning) of the contralateral EC-DG response more severely retards CR aquisition (i.e., contralateral potentiation) then simple UCS preexposure. Contralateral then ipsilateral stimulation pairing, with no preexposure, increases the contralateral response 71%, with the potentiation reaching an apparent asymptote relatively quickly. If ipsilateral conditioning stimulation precedes paired conditioning (UCS preexposure), there is only a 60% increase in the contralateral response, with a slightly slower growth in response size. Prior backward pairing (UCS then CS) of ipsilateral and contralateral conditioning stimulation, followed by normal contralateral-ipsilateral pairing, produces a 43% increase, attaining this final level much more slowly than the other two paradigms. (N=10, all groups). Thus, just as found in CC of behavioral responses, the EC-DG analogues of UCS preexposure and backward conditioning preexposure sharply attenuates associative synaptic modification. Although the synapses studied here do not mediate CC of the intact behaving animal, we hypothesize that the qualitative properties of the EC-DG synapses exist at synapses subserving the associative encoding of CC. In particular, the preacquisition effects examined here come from synaptic rather than circuit properties. [1]Siegel & Domjam. Learn.& Motiv. 2(1971)1. [2]Levy & Steward. Br.Res. 175(1979)233. [3]Levy & Steward. Neurosci.8(1983)799. Supported by AFOSR 83-0236 & NS15488 to WBL.

245.4 THE EXPRESSION OF LEARNING-INDUCED PLASTICITY OF SINGLE NEURONS IN THE AUDITORY CORTEX IS CONTEXT-DEPENDENT. D.M. Diamond and N.M. Weinberger. Center for the Neurobiology of Learning and Memory and Dept. of Psychobiology, U.C. Irvine, Irvine, CA 92717

Previously, we have shown that classical conditioning induces evoked plasticity in the auditory cortex of the cat. Specifically, the single neuron discharges evoked by an acoustic conditioned stimulus (CS) are altered during acquisition of the pupillary dilation conditioned response in 95% of neurons recorded in the secondary field (Beh. Neurosci., 98:189-210). In order to characterize the degree of specificity of such plasticity, we studied aspects of the receptive field properties of auditory cortical neurons by obtaining frequency response functions to isointensity tones before and after behavioral conditioning for each neuron. Evoked plasticity was often specific to the frequency of the CS, rather than reflecting associatively induced general changes in responsivity to other stimuli in the tuning function (Neurosci. Abstr., 10:245). The present report is an analysis of the relation between the manner in which cells develop evoked plasticity on single trials during conditioning and the expression of plasticity in frequency tuning functions.

Details of the procedures are presented in Neurosci. Abst. 10:245. Given that learning produces CS frequency-specific plasticity, it might be assumed that the effect is fixed, i.e., the cell is now excited either more or less by the CS frequency under all circumstances. An alternative is that learning alters the processing of the CS frequency in a manner which is not fixed, but rather is sensitive to the context in which this stimulus is presented. To resolve this issue, we compared the direction of learning induced change to the CS during single trials of conditioning with the response to the frequency of the CS when it was presented within a sequence of tones used to obtain tuning functions. A total of 19 cells were analyzed, of which 100% developed evoked plasticity on either or both single trials and tuning functions. The expression of plasticity was not the same in most cases (13/19).

These findings indicate that the effects of learning upon discharge plasticity in the auditory cortex cannot be characterized simply as an increase or decrease in response to the frequency of the CS, but also reflect the context within which the stimulus is presented. This strongly suggests that the expression of learning-induced evoked plasticity reflects processes that are related to a neural network in which individual components have a differential influence on cortical neurons depending on the context of stimulation.

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245.5 EYEBLINK CONDITIONING USING LRN STIMULATION AS A CS IS ABOLISHED BY LESIONS OF THE CEREBELLAR NUCLEI. B.J. Knowlton, D.G. Lavond, J.E. Steinmetz & R.F. Thompson. Dept of Psychology, Stanford Univ, Stanford, CA 94305.

Discrete lesion of the cerebellar interpositus nucleus abolishes or prevents learning of classically conditioned somatic responses without affecting the unconditioned response. This observation has led to examination of afferents to the interpositus as possible CS pathways. The lateral reticular nucleus (LRN) has extensive projections as mossy fibers to the interpositus and cerebellar cortex. In the present study direct stimulation of the LRN was used as a CS in order to examine a) its efficacy as a CS, b) the dependence of the learned response on the cerebellar deep nuclei, and c) the degree of similarity with tone as a CS.

Adult male New Zealand White rabbits were anesthetized and implanted with bipolar stimulating electrodes in the LRN. Training consisted of 108 trials per day (30 sec mean ITI) in which the CS (LRN stimulation, 200 Hz, 350 ms, 60-300 uA) was paired with a coterminating UCS (corneal airpuff, 2.1 $N/cm^2$, 100 ms). A total of 18 rabbits received paired training and overtraining (1-3 days) followed by CS-alone extinction (4 days) and unpaired CS and UCS trials (2 days). CS-alone and unpaired training were given to evaluate the possibility of sensitization. Seven rabbits were then given ipsilateral lesions of the dentate-interpositus nuclei, allowed recovery, and given paired training (5 days). These rabbits were then trained with the air on the opposite eye (typically 2 days). Another 4 rabbits received the CS-alone and unpaired exposures before paired conditioning and overtraining.

All of the rabbits with placements near or in the LRN learned regardless of the order of training. The mean number of trials to criterion (8 CRs in 9 consecutive trials) was 79.5. (Training normally requires over 100 trials with tone as a CS.) Complete interpositus lesions abolished the CR with no recovery (N=5); relearning occurred with incomplete lesions (N=2).

The present study demonstrates that a major cerebellar afferent can act as an effective CS and that the conditioned response associated with this training must use cerebellum. Current studies also demonstrate that stimulation of the dorsolateral pontine nucleus can act as an effective CS, and that classical conditioning occurs with paired mossy fiber (CS) and climbing fiber (UCS) stimulation. We suggest that learning/plasticity occurs within the cerebellum.

Supported by NSF predoctoral fellowship to BJK and by ONR contract N00014-83-K-0238, NSF grant BNS-81-17115 and a grant from the Sloan Foundation to RFT.

245.6 SHORT-DURATION MOSSY FIBER STIMULATION IS EFFECTIVE AS A CS IN EYELID CLASSICAL CONDITIONING. C.G.Logan*, J.E.Steinmetz, D.S. Woodruff-Pak and R.F.Thompson (SPON: M.E.Stanton). Dept. of Psychology, Stanford University, Stanford, CA 94305.

It has recently been demonstrated that electrical stimulation of mossy fiber input to the cerebellum can serve as an effective conditioned stimulus (CS) in classical conditioning of the rabbit eyelid response (Steinmetz, Lavond & Thompson, Bull. Psychon. Soc., 1985). Electrical stimulation of the dorsolateral pontine nucleus (DLPN), when paired with a corneal airpuff unconditioned stimulus (US) in a standard delay paradigm (350 msec CS overlapping in the last 100 msec with the US), results in rapid and robust learning. In such trained animals, reducing the stimulation duration to 12 msec is sufficient to sustain conditioning. The purpose of the present study was to determine whether a short duration (21 msec) stimulation would also be sufficient for acquisition.

Male New Zealand white rabbits were chronically implanted with bipolar stimulating electrodes in the right DLPN and with a recording electrode in the left hippocampus. Following recovery,animals were trained to a criterion of 8/9 CRs with a 21 msec train of DLPN stimulation CS (90-120 uA, .1 msec, 200 Hz), paired with a 100 msec corneal airpuff US beginning 250 msec after CS onset. Thus, there was a 229 msec period between the offset of the CS and the onset of the US. Animals were trained to criterion, overtrained, retrained with a 350 msec tone CS, and then extinguished with unpaired DLPN stimulation-airpuff presentations.

Rabbits required an average of 261 trials to reach criterion with the short duration CS. This is a somewhat greater number of trials to criterion than was required for animals given training with 350 msec DLPN stimulation. Transfer to tone was relatively rapid, and explicitly unpaired presentation of CS and US produced extinction within 5 days in all cases. Such extinction argues against the possibility that sensitization or some other non-associative process was responsible for the original learning.

These data demonstrate that a brief duration CS is sufficient for acquisition of the conditioned eyelid response and suggest that CS onset and stimulus onset asynchrony may be more significant than CS duration for acquisition of the conditioned response. In addition, this preparation allows recording of brain activity after delivery of a stimulation CS.

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245.7 CLASSICAL CONDITIONING OF THE RABBIT EYEBLINK DOES NOT OCCUR WITH STIMULATION OF THE CEREBELLAR NUCLEI AS THE UNCONDITIONED STIMULUS P. F. Chapman*, J. E. Steinmetz, & R. F. Thompson (SPON: L. Holt). Department of Psychology, Stanford University, Stanford, CA 94305.

Recently, the cerebellum has been shown to be essential for the development and expression of the classically conditioned eyeblink response in the rabbit. Lesions of the cerebellar nuclei (medial dentate-lateral interpositus) abolish the conditioned response (CR) but not the unconditioned response (UR) (McCormick and Thompson, J. Neuroscience, 4, 1984). In addition the red nucleus (RN), a primary efferent of the cerebellum, has been shown to be a necessary part of the conditioned response pathway (Haley et al, Neuroscience abstracts, 1983). Furthermore, activation of climbing fibers as the unconditioned stimulus (US) via stimulationt of the dorsal accessory olive (DAO) produces conditioned responses identical to those using the standard air puff US (Mauk & Thompson, Neuroscience Abstracts, 1984) and lesions to the DAO cause extinction of the learned response (Steinmetz et al, Neuroscience Abstracts, 1984) Since the DAO is known to be an element of the US pathway, and the cerebellum and RN are elements of the CR pathway, plasticity must occur at the cerebellum, or at an efferent point. Stimulation along this pathway afferent to the site of plasticity should produce normal learning, as with DAO stimulation, whereas stimulation efferent to this site will produce the UR, but not the CR. In this study, male albino

Rabbits were trained using stimulation of the dentate-interpositus (DI) as the US and a tone as the conditioned stimulus (CS). US stimulation consisted of 100 msectrains 0.1 msec cathodal pulses, ranging in frequency from 200-400 Hz, and in intensity from 50 to 500 uA. Stimulation elicited a variety of responses ranging from lateral head movements and front or hind leg movement to eyeblinks. Those animals exhibiting eyeblinks were tested for five days (108 trials per day) using the standard eyeblink paradigm, with a 250 msec interstimulus interval. Animals displayed robust URs, but developed no CRs during the five days of training. After the stimulation training, animals were switched to standard training, using corneal air puff as the US. Within 108 trials, all animals had reached a criterion 8 CRs in 9 consecutive trials, indicating that their inability to learn using the stimulation US was not due to damage to the DI or any interference with the tone perception.

These results suggest that the learning related plasticity that occurs in the conditioned eyeblink response is not efferent to the cerebellum.

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245.8 Neuronal Responses of the Rabbit Cerebellar Cortex During Performance of the Classically Conditioned Eyelid Response. Nelson H. Donegan, Michael R. Foy, and Richard F. Thompson. Department of Psychology, Stanford University, Stanford, CA 94305

Recent work has shown that the cerebellum is an essential element of the neural circuitry responsible for the development and expression of the conditioned eyeblink response in the rabbit (e.g., McCormick & Thompson, 1984). Multiple-unit recordings in the deep cerebellar nuclei and the overlying cerebellar cortex have revealed areas in which neuronal activity precedes and models the learned behavior. In the present study, single-unit recordings were made in the ansiform cortex of the cerebellum ipsilateral to the side of training in order to characterize the types of neuronal responses exhibited by this region to the various components of the trial events (tone CS and airpuff US) and the behavior exhibited on the trial (CR and UR). During the course of the behavioral training and recording sessions, subjects received pairings of an acoustic CS (350 msec, 1 kHz tone) with a corneal airpuff US (100 msec, which overlapped and terminated with the CS). The response measure was EMG activity recorded from the upper eyelid. Recording sessions were conducted after the subjects showed consistent CRs to the tone CS. Thus the majority of the neurons sampled were analyzed when subjects were well trained.

Subsequent analysis of single-unit activity revealed several classes of neural responses correlated with behavior: Units that (1) showed a burst of activity at the onset of the conditioned response and a high level of responding over the course of both the CR and UR, (2) showed inhibition at the onset of the conditioned response and throughout responding, (3) showed increases in activity that preceded and modelled the CR. In some cases the cells could be identified as Purkinje cells because of complex spikes characteristic of climbing fiber input.

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245.9 RESPONSES IN MEDIAL HABENULA DURING RABBIT NM CONDITIONING. R.A. Swain* and S.D. Berry. Dept. of Psychology, Miami Univ., Oxford, Ohio 45056.

Several areas of the limbic forebrain show significant neural responses during classical conditioning of the rabbit nictitating membrane (NM) response. An important question that remains, however, is how such activity is relayed to brainstem motor systems that underlie the reflex and conditioned responses. While conditioned neural responses have been described in the hippocampus and lateral septum, further tracing of subcortical outputs has failed to show significant neural responses in hypothalamic targets of septal or hippocampal output. An additional septo-hippocampal output pathway has been traced through the medial habenular nucleus which receives a major projection from the posterior septal region and projects to the interpeduncular nucleus via the fasciculus retroflexus (Herkenham & Nauta, J. Comp. Neur.,173, 1977; Swanson & Cowan, J. Comp. Neur.,186, 1979). In this paper we report significant increases in medial habenular unit activity during rabbit NM conditioning.

New Zealand White rabbits were anesthetized with Ketamine and implanted with stainless steel extracellular recording electrodes in the medial habenular nucleus and CA1 layer of the hippocampus. Animals were allowed 1 week recovery prior to conditioning. Training consisted of 26 blocks of trials, with each block consisting of 8 paired and 1 CS alone test trial. Each paired trial consisted of a 350msec tone (1KHz,85dB) followed after 250msec by a 100 msec corneal air puff (210g/cm2). Control animals were given explicitly unpaired tone and air puff presentations. A CR was defined as at least 1/2 mm of NM extension prior to air puff onset. Neural responses were averaged over the 8 trials of each block and are displayed as poststimulus histograms. In addition, standard scores were computed to quantify responses across blocks.

Initial results indicate a significant habenular response to the UCS (air puff) that appears, like hippocampal and septal responses, to precede and model the behavioral response. The average increase in habenular firing in response to the UCS was greater than 4 standard scores, indicating a significant increase over baseline levels. Figure 1 shows a representative histogram from a medial habenular recording. Arrows indicate tone (left) and air puff (right) onset.

These results suggest that forebrain limbic responses during NM conditioning may be projected to the brainstem via the habenula and its efferents.

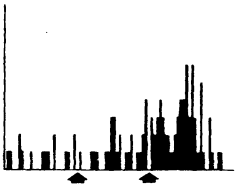

Figure 1

245.10 EFFECTS OF SEPARATE AND COMBINED MEDIAL AND ANTERIOR THALAMIC LESIONS ON DISCRIMINATIVE AVOIDANCE LEARNING IN RABBITS. M. Gabriel, S.P. Sparenborg, P. Colletier*, and J. Tenner*. Dept. Psychol., Univ. Illinois, Champaign, IL 61820

The experiment reported here is one of an ongoing series in which the discriminative avoidance behavior of rabbits is used as a model system for study of the neural mechanisms of learning. Past studies of the neuronal activity of the posterior cingulate cortex (area 29) and the anteroventral nucleus (AVN) of thalamus have suggested that these structures are important substrates of the behavior (Gabriel et al., Science, 208: 1050, 1980). Yet, the effects of damage in area 29 or in the AVN were rather minimal, each lesion yielding a significant but only partial impairment of the performance of the well-learned behavior, and no effect on its original acquisition (Gabriel et al., Behavioral Neuroscience, 97: 675, 1983; Ragsdale et al., Neurosci Abstr., 1984). These results suggested that area 29 and the AVN partially subserve performance of the well-learned behavior but that there exists an additional neural system for the mediation of acquisition.

The system comprised by the anterior cingulate cortex (areas 24 and 32) and the mediodorsal thalamic nucleus (MDN) represents a plausible candidate for acquisition, given the very rapid development of associative correlates of training in these structures (Orona et al., Brain Research, 263: 295, 1982). Here we report that bilateral electrolytic lesions of the MDN which spared the AVN ($N = 4$) were associated with a retardation of acquisition ($P<.05$) and impaired asymptotic avoidance performance ($P<.05$) relative to unlesioned controls ($N=10$). Subjects with partial lesions ($N = 4$) in AVN and MDN were not different from controls, but lesions that encompassed more than 50% of AVN and MDN bilaterally ($N=6$) yielded a severe acquisition deficit. None of the subjects in the combined lesion group (but all of the subjects in the control, partial and individual lesion groups), attained the acquisition criterion. In addition, the combined but not the separate lesions abolished all cue-elicited excitatory and discriminative neuronal activity in the cingulate cortex during training (Gabriel et al., Neurosci Abst, 1985). These data suggest that the MDN and the AVN, and their respective cingulate cortical subfields comprise a pair of neural systems that are to a degree functionally specific, respectively, for mediation of original acquisition and maintenance of the well-learned behavior. Yet these systems also manifest functional redundancy in that each contributes substantially to the specialty of the other. Together they comprise a supersystem the totality of which is essential for the learned behavior.

245.11 SEPARATE AND COMBINED MEDIAL AND ANTERIOR THALAMIC LESIONS AND CINGULATE CORTICAL NEURONAL CORRELATES OF AVOIDANCE LEARNING IN RABBITS. P. Colletier*, J. Tenner*, S.P. Sparenborg, and M. Gabriel. (SPON: P. Johnston). Dept. Psychol., Univ. Illinois, Champaign, IL 61820

Past studies have indicated that experimental damage in the anterior thalamic nuclear group, or in the related posterior cingulate cortical subfield (area 29), is associated in rabbits with a decrement in the postasymptotic performance of a discriminative avoidance habit, but such damage does not affect original acquisition to behavioral asymptote (Gabriel et al., Behavioral Neuroscience, 97: 675, 1983; Ragsdale et al., Neurosci Abstr., 1984). In contrast, damage in the mediodorsal thalamic nucleus (MDN) is associated with impaired behavioral acquisition, and combined anterior and medial thalamic damage devastates acquisition (Gabriel et al., Neurosci Abst., 1985). Taken together, these results suggest that the medial and anterior thalamic nuclei and their respective cingulate cortical subfields contribute to the avoidance behavior in a parallel and somewhat redundant fashion, and that both systems together comprise a supersystem that is essential for the behavior.

If the idea of parallel and redundant processing as applied to these neural systems is valid then it should be possible to demonstrate continuing operation of one of the systems when the other is disabled. This expectation was supported by past results indicating that anterior thalamic lesions abolished all excitatory and discriminative neuronal activity normally elicited in area 29 by the conditional stimulus (CS+) during performance of intact rabbits, but such lesions did not block the development of discriminative neuronal correlates of training in the anterior cingulate cortex (areas 24 and 32, op cit., 1983). Here we provide further support for the idea of parallel and redundant contributions of the two systems. Bilateral electrolytic lesions confined to the mediodorsal thalamic nucleus (MDN) eliminated the excitatory and discriminative neuronal correlates of training in area 24, but the neuronal correlates in area 29 survived such lesions. Also, consistent with their devastating behavioral effects, combined anterior and medial thalamic lesions eliminated the excitatory and discriminative neuronal activity in both of the cingulate cortical subfields.

245.12 SHORT-LATENCY NEURONAL DISCHARGES IN THE RABBIT CINGULATE CORTEX PREDICT THE BEHAVIORAL OUTCOME OF TRIALS DURING DISCRIMINATIVE AVOIDANCE CONDITIONING. S.P. Sparenborg and M. Gabriel. (SPON: W.T. Greenough). Dept. Psychol., Univ. Illinois, Champaign, IL 61820

Past studies have revealed that CS-elicited neuronal activity in the cingulate cortex and in the related nuclei of the thalamus becomes discriminative during the course of avoidance conditioning in rabbits. That is, the frequency of action potentials elicited by a tone (CS+) predicting the occurrence of the footshock UCS was greater than that elicited by the tone (CS-) predicting no occurrence of the UCS. We have interpreted the discriminative effects as representing processes involved with the encoding of the differential associative significance of the CSs, but it is possible as well that the effects, even at brief latencies, may covary directly with the behavioral outcomes of the conditioning trials. In order to explore this possibility separate peristimulus histograms were constructed for each of four trial types; a) CS+ followed by a conditioned response (CR), b) CS+ followed by no CR, c) CS- followed by a CR, and d) CS- followed by no CR. The rabbits were trained to locomote in an activity wheel to avoid a footshock UCS whenever they heard the CS+ and they learned to ignore the CS-. Sixty trials daily with each stimulus were presented in a random order until a criterion of discriminative avoidance performance was attained . The four-fold classification of the trials was carried out for 29 cingulate cortical and 18 anteroventral thalamic neuronal records obtained from the session of the first significant behavioral discrimination and for the criterial session.

The results indicated that two distinct aspects of behavioral performance could be predicted on the basis of the average histograms for the first 200 milliseconds following CS onset. As in all past studies, the average CS-elicited activity in cingulate cortex manifested an inhibitory pause measured relative to the pre-CS baseline at 40 - 60 milliseconds after CS onset, and an excitatory discharge from 100 to 400 milliseconds after CS onset. The activity on CS- trials followed by response manifested no inhibitory pause and thus a significant elevation, relative to the activity from 40 - 60 msec in each of the remaining trial types. In addition, the activty elicited by the CS+ on trials with CRs was significantly greater than the activity on CS+ trials without CRs, in the interval from 100 - 200 milliseconds. These effects were restricted to records obtained from the deep laminae (5&6) of the posterior cingulate cortex.

245.13 CONDITIONING-INDUCED WAVEFORMS RECORDED OVER OLFACTORY AND PARIETAL AREAS OF RAT BRAIN. L.M. Grimes* and R.S. Dyer (SPON: M.I. Gage). Northrop Services, Inc. (LMG) and Neurophysiol. Branch, NTD, HERL, USEPA (RSD), Research Triangle Park, NC 27711

The properties of early components of sensory evoked potentials recorded from the scalp of humans and nonhumans are intimately tied to the physical properties of the evoking stimulus. Later components have been linked to cognitive events in humans, nonhuman primates and cats, and may reflect stimulus significance and/or other cognitively applied attributes. The present series of studies explored the possibility that changing the significance of an auditory stimulus might alter the later components of tone evoked potentials in rats.

Skull screw (00-90) electrodes were implanted at sites ranging from 25 mm anterior to 6 mm posterior to bregma, and from the midline to 4 mm lateral. All recordings were referred to a screw over the cerebellum. Auditory evoked potential averages were obtained following 150 presentations of a 10 kHz tone (30, msec, 85 dB SPL) before and 3 hrs after pairing of the tones with footshock (conditioning). The conditioning paradigm consisted of 30 presentations of the tone, followed 500 msec later by a 2 mA, 750 msec footshock. Control rats received footshocks not paired with tones. In conditioned rats, two apparently separate late responses occurred at different electrode sites. One response, largest overlying the cribriform plate (bregma + 10 mm), was a negative wave with a peak latency of about 250 msec, and an amplitude of about 60 uV. Blocking entry to the nose induced mouthbreathing and diminished the response, while removing the blockage reinstated the response. These results suggest that the response may be due to a conditioned breathing (inspiratory) response, perhaps bringing odor molecules in contact with the sensory epithelium of the nose and thereby eliciting a potential akin to Ottoson's. The other response appeared greatest over a parietal site (bregma-3 mm, lateral 3 mm), and was manifested by a biphasic negative (190 msec)-positive (290 msec) waveform with an amplitude of about 40 uV. Both the olfactory and parietal waveforms were eliminated by halothane and ketamine anesthesia. The role (if any) played by stimulus induced movement in the potentials recorded from conditioned animals remains to be determined, as does the relationship between these waveforms and those previously reported in other species.

245.14 DIFFERENTIAL RESPONSE OF A SELECT POPULATION OF PREFRONTAL CORTEX NEURONS TO REINFORCED AND UNREINFORCED CONDITIONED STIMULI. S.L. Peterson. Dept. Medical Pharmacology, Texas A&M Univ., College Station, TX 77843.

Rats were prepared with chronic intracranial implants for the subsequent (7-10 day) recording of the extracellular action potentials from prefrontal cortex neurons using tungsten microelectrodes (9-11 M ). During the recording experiments the animals were unanesthetized, unparalyzed and suspended in a bodysling with their heads fixed in position by way of the intracranial implant. The animals were conditioned to the stimuli prior to the recording experiment. The conditioned stimuli used were high (3000 Hz) or low (400 Hz) pitch tones of 2 sec. duration . The reinforced stimuli (CS+) were followed immediately by a mild, 10 msec. duration electric shock to the hindpaws. The unreinforced stimuli (CS-) were presented unpaired and were always represented by the same tone in an individual animal. Animals had been conditioned to the stimuli prior to the recording experiment.

Only neurons with characteristic large amplitude, positive first deflection action potentials were evaluated (Pirch et al. Exp. Neurol. 82:118, 1983). These select action potentials probably represent cortical pyramidal neurons as related experiments in chloral-hydrate anesthetized rats demonstrate that some of the neurons can be antidromically activated from the ventral tegmental area.

The neuronal activity during the randomly presented CS+ and CS- was compiled in computer generated peri-stimulus time histograms and compared by paired t-test. Neurons in the deeper prefrontal cortex layers (n=5) emitted significant differential responses to the CS+ and CS- which extinguished over 200-300 stimuli when the footshock was withheld. Other neurons in the deeper layers (n=6) also emitted significantly different responses but could not be tested for a sufficient period of time to demonstrate extinction. Neurons in superficial prefrontal cortex layers (n=5) did not emit differential responses.

Since the deeper layers of the prefrontal cortex are innervated by dopamine while the superficial layers are not, this data suggests that only the prefrontal cortex neurons which are innervated by dopamine emit differential responses to reinforced and unreinforced conditioned stimuli. These observations provide evidence for a functional relationship of the reciprocal innervation between the dopaminergic ventral tegmental area and the prefrontal cortex.

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245.15 SELECTIVE CHANGES IN SYNAPTIC RESPONSES ELICITED IN A CORTICAL NETWORK BY BEHAVIORALLY RELEVANT ELECTRICAL STIMULATION. U. Staubli, F. Roman and G. Lynch* (SPON: J. Conway). Center for the Neurobiology of Learning α Memory, Univ. of California, Irvine, CA 92717.

The goal of this project was to develop a paradigm for studying the possibility that experience selectively modifies synapses in cortical networks. The olfactory modality was chosen as model system since its primary target area (olfactory cortex) offers the advantage of being organized along well defined anatomical lines with simple but nonetheless cortical properties.

Rats were implanted with 3 bipolar stimulating electrodes aimed at different locations of one olfactory bulb. A single recording electrode was positioned in the dendritic field of the stellate cell layer of the piriform cortex so that biphasic pulses (10-150μA) to each stimulation pole elicited a negative field potential ranging between 1-3 mV in amplitude. The rats were then trained on successive days in an operant chamber to break a light barrier for water reward in the presence of a designated odor, but not to respond to a second odor (a buzzer signalled a wrong response). Asymptotic performance was reached after 3 successive 2-odor discriminations, using a different odor pair each day. (We have found that rats remember both the positive and negative odors in a series of pairs for weeks after this type of training). At this point the natural odors were replaced by electrical stimulation of the olfactory bulb: We found that 4 pulses separated by 10ms given at the frequency of sniffing (6 Hz) were effective in eliciting sniffing, rearing and searching behavior. Duration of stimulation varied between 3s and 15s per trial, with ITIs of at least 30s. The same type of patterned stimulation, but of a different part of the bulb, was used as 'negative' cue and was applied in random order with the 'positive' cue. The rats acquired this type of discrimination rapidly (within 15 min). Evoked responses to single pulses were measured before, immediately and at several time points after the learning session. Stimulation intensity applied to the various poles was identical to that used during patterned stimulation. The responses (20 per pole and time point) were averaged and the slope and amplitude calculated. Comparisons of these measures revealed a substantial increase (up to 160%) after learning in case of the 'positive' and 'negative' electrodes, whereas the potentials evoked by the neutral electrode (no stimulation during the learning session) remained unchanged. The increment of the evoked responses remained stable for at least several days after training (3 weeks being the longest test interval used) as was the memory of the discrimination. These data provide preliminary evidence of long-lasting synaptic plasticity in the bulbar-piriform projections and suggest that this system may be useful in studying the effects of experience on the physiological properties of a well-defined collection of cortical circuitries. (supported by an ONR Grant Δ N00014-84-K0391)

245.16 **NOVEL AND AVERSIVE CHEMOSENSORY STIMULI: DISCRIMINATION BY THE RAT FETUS IN UTERO.** W.P. Smotherman and S.R. Robinson* Laboratory for Psychobiological Research, Department of Psychology, Oregon State University, Corvallis, OR 97331.

Rat fetuses can form conditioned taste/odor aversions in utero as early as Day 17 of gestation. They exhibit reduced activity in utero when reexposed to the aversive CS on Day 19. This conditioned response comprises a similar number and patterning of fetal movements to the behavior observed on the day of conditioning (Smotherman and Robinson, Behavioral Neuroscience 99: 521-530, 1985).

We have further characterized the responsiveness of rat fetuses to a conditioned taste/odor stimulus in utero. As in the original experiments, fetuses were exposed to a mint solution (100%) via intra-amniotic injection on Day 17 followed by ip injection of lithium chloride (LiCl). Two days later, on Day 19, the pregnant female was surgically prepared by chemomyelotomy and placed in a temperature-controlled saline bath to allow direct observation of fetal behavior. Subject fetuses were externalized into the bath, maintaining intact the placental-uterine attachment. While remaining within it's amniotic sac, each fetus was exposed to one of five chemosensory test stimuli: saline, 100% mint (the original mint solution), 50% mint, 200% mint, or a novel solution of lemon extract. Following stimulus presentation, the behavior of each fetus was observed.

The results of this experiment replicate and extend the findings of our original conditioning study. Fetuses presented with a pairing of 100% mint and LiCl on Day 17, when reexposed to 100% mint on Day 19, responded with markedly suppressed activity. Fetal responses to 200% mint were indistinguishable from responses to 100% mint, with reduced frequency of general activity and complex movements as well as fewer individual movements of the forelegs, hindlegs and head. An intermediate level of suppression in general activity and individual movements was evident for the 50% mint solution. But the aversive response of fetuses was specific to mint; saline and lemon solutions elicited similar levels of general fetal activity and lemon actually activated fetal foreleg movements. Three inferences may be drawn from these results. First, fetuses are capable of forming and exhibiting learned behavior in utero. Second, fetuses are capable of discriminating between pairs of chemosensory stimuli, one which is aversive and the other neutral. Third, fetuses may be capable of discriminating between different concentrations of the same taste/odor stimulus.

This research is supported by Grant HD 16102-04 from the National Institute of Child Health and Human Development to WPS.

245.17 THE IDENTIFICATION AND PARTIAL CHARACTERIZATION OF A CELL SURFACE PROTEIN THAT MODULATES REM IN NEONATAL RATS AND LONG TERM SYNAPSE PLASTICITY IN HIPPOCAMPAL SLICE PREPARATIONS. J.R. Moskal*[1], A.E. Schaffner[2] and K.J. Koller[1], Lab. Cell Biol., NIMH[1] and Lab. Neurophys., NINCDS[2], Bethesda, MD 20205.

A monoclonal antibody (B6E11), derived from a panel generated against 5-day postnatal rat dentate gyri, was found to significantly increase REM when injected intrahippocampally into neonatal rats. It was also found to block the formation and maintainance of long term potentiation in hippocampal slice preparations without any effect on normal synaptic transmission. Another antibody (G6E3) from the same panel, same immunoglobulin class ($IgG_1$) and with similar immunohistochemical properties had no effect on these processes. Immunocytochemical staining revealed that B6E11 recognized only dentate gyrus granule, hippocampal pyramidal and cerebellar Purkinje neurons in unfixed frozen sections of adult rat brain. That B6E11 recognizes a cell surface antigen is based on radioimmunoassays with crude membrane fractions, flow cytometric analysis of unfixed dissociated cells from rat brain and the ringed fluorescence found on neurons in primary hippocampal cultures. Immunoblot analysis of hippocampal homogenates subjected to SDS-PAGE revealed that B6E11 recognized a developmentally regulated protein that increases markedly in concentration (per mg of protein) from birth through adulthood and changes in molecular weight from $M_r$=37,000 at birth through 5 days postnatal followed by a doublet of $M_r$=37,000 and $M_r$=42,000 until 20 days postnatal and thereafter is found as a single band of $M_r$=42,000. This protein is also found in adult rhesus monkey hippocampus and is soluble in 2% Triton X-100. Neither B6E11 nor G6E3 were able to inhibit the specific binding of radiolabeled glutamate or kainic acid to either hippocampal or cerebellar membranes. Further experiments are in progress to determine if this antigen is involved in the regulation of a specific receptor or ion channel.

245.18 EFFECTS OF A MONOCLONAL ANTIBODY AGAINST RAT HIPPOCAMPAL DENTATE CELLS ON REM ACTIVITY IN FIVE-DAY OLD RAT PUPS. R.A. Deyo*, R. Conner*, J. Panksepp, and J.R. Moskal*[1] (SPON: K. Koller). Dept. Psychology, Bowling Green State Univ., Bowling Green, OH, 43403, and [1] Lab. of Cell Biology, NIMH, Bethesda, MD 20205.

A series of monoclonal antibodies was generated to the dentate gyrus of five-day old rat pups. Several of these antibodies have been systematically screened for their possible behavioral effects. Results obtained with one antibody, designated B6E11, are herein reported.

In young rat pups, rapid eye movements (REM) are known to correlate highly with leg twitches. We tested 12 five-day old rat pups and found a correleation between REM and leg twitches to be r=0.9799. In a study with 56 five-day old rat pups, the animals were randomly assigned to one of four treatment groups (n=14): (1) active anti-hippocampal antibody B6E11; (2) a second anti-hippocampal antibody G6E3; (3) 0.9% saline solution; or (4) a no-injection group. Animals in groups 1-3 were injected with 1-2 µl bilaterally into the dorsal hippocampus with the appropriate solution. Monoclonal antibodies in pooled ascites fluid were diluted 1:20 in 0.9% saline, or affinity purified by protein A-Sepharose chromatography, and approximately 0.1-1.0 µg of antibody was injected. Leg twitches were then recorded during four successive blocks of 7 min. each. Analysis of variance revealed a significant treatment effect (F=5.05, with 3.52 d.f., p=0.0038). Post-hoc comparisons (Tukey's q-statistic, alpha=0.05) revealed that animals treated with B6E11 had significantly more leg twitches (mean =9.03) than animals treated with G6E3 (mean=5.50), 0.9% saline (mean=5.25), or non-injected animals (mean=5.39). Both B6E11 and G6E3 are $IgG_1$'s, and both recognize only hippocampal pyramidal, dentate gyrus granule, and cerebellar Purkinje neurons in frozen sections of adult rat brain. These antibodies also stain living neurons in primary hippocampal cultures suggesting that they recognize cell surface antigens.

These results demonstrate that the monoclonal antibody, B6E11, specifically elevates REM when injected intrahippocampally. Overall, these results suggest that monoclonal antibody techniques might be utilized to identify the antigen or antigens involved in REM mechanisms.

245.19 A MONOCLONAL ANTIBODY (MAb) TO A HIPPOCAMPAL CELL-SURFACE ANTIGEN WHICH INHIBITS BOTH PRODUCTION AND MAINTENANCE OF LONG-TERM POTENTIATION (LTP) IN RAT HIPPOCAMPAL SLICES. P.K. Stanton, J.M. Sarvey, and J.R. Moskal*[1]. Dept. Pharmacology, Uniformed Services University of the Health Sciences, and [1]Lab of Cell Biology, NIMH, Bethesda, MD

LTP is a long-lasting enhancement of evoked responses after high-frequency afferent stimulation, which has become a popular model of neuronal plasticity and candidate memory mechanism. We have recently shown that protein synthesis inhibitors impair LTP production, and others have shown correlative changes in cell morphology, protein synthesis and phosphorylation. However, little is known of the loci or nature of specific macromolecules required in LTP. Therefore, MAb's were made to 5-day postnatal rat dentate gyri, screened for their ability to recognize cell-surface antigens in the hippocampal formation, and tested for effects on hippocampal responses and LTP. One MAb, called B6E11, impaired production of LTP and suppressed established LTP in hippocampal slices, without long-term effects prior to high-frequency stimulation.

Hippocampal slices (400µm thick) were cut from adult rats (150-300 g) and two extracellular recording sites 200-400 µm apart in somatic or dendritic layers of the dentate or CA1 were chosen. Stimuli were given via perforant path or Schaffer collateral axons respectively. One of two recording sites was randomly chosen for MAb application, the other as control, and MAb pressure-applied near either somas or dendrites of the MAb site. MAb (~0.2-0.6 µg) was ejected (100-200 µm dia., 2-3 times 1 min apart) 15 min before stimulation, and LTP measured 30-min post-stimulation.

Control LTP(% of pre-stimulated spike amplitude) in dentate was 167 ± 9.0%(N=10). In contrast, B6E11-treated somatic and dendritic sites in dentate exhibited marked impairment of LTP: B6E11 soma = 97 ± 6.6%; B6E11 dendrites = 105 ± 14.2% (both $p<.05$, N=7,3,paired t-test). Furthermore, B6E11 applied to cell somas 30 min after high-frequency stimuli suppressed established LTP: Control LTP = 219 ± 22.1%, B6E11 sites = 136 ± 18.4% ($p<.05$, N=5,paired t-test). In contrast, control experiments with another MAb (G6E3) from the same panel, the same immunoglobulin class ($IgG_1$) and similar immunohistochemical properties as B6E11, showed no inhibition of LTP.

In field CA1, where synapses are on apical dendrites further from the cell soma than in the dentate, B6E11, when applied to cell somas, did not impair LTP: Control LTP = 193 ± 22.8%, B6E11 sites = 190 ± 31.5% (N=4). However, B6E11 application to CA1 pyramidal cell dendrites significantly inhibited LTP production: Control LTP = 221 ± 28.9%; B6E11 sites = 124 ± 9.1% ($p<.05$, N=6, paired t-test), and also suppressed established LTP: Control LTP = 129%, B6E11 sites = 98% (N=2).

These results indicate the presence of a cell-surface antigen which, properly expressed, plays a key role in both production and maintenance of LTP. Furthermore, the data in CA1 supply direct evidence that this antigen functions primarily in cell dendrites.

246.1 ONTOGENETIC CHANGES IN THE Na AND H GRADIENTS ACROSS BASOLATERAL MEMBRANE OF CHOROID PLEXUS: A CLUE TO THE MATURATION OF THE CSF SECRETORY PROCESS. C.E. Johanson, Dept. of Pharmacology, Univ. of Utah Sch. Med., Salt Lake City, UT 84132.

Exchange of intracellular hydrogen ($H_i$) for extracellular sodium ($Na_o$), i.e., Na/H antiport, in the basolateral (plasma-facing) membrane of choroid plexus (CP) is thought to be the primary system for moving Na from interstitial fluid into CP epithelium, i.e., the rate-limiting step in CSF formation (Murphy, Ph.D. thesis, 1984, Univer. of Utah). Aronson demonstrated that 1:1 Na/H exchange depends on $[Na]_i/[Na]_o$ (i.e., rNa) being >rH to allow Na and H transport, respectively, into and out of the cell.

The aim has been to analyze cation gradients across in vivo CP basolateral membrane throughout postnatal development; and to relate them to established information about tracer-Na movement across the blood-CSF barrier and CSF secretory capacity. Sprague-Dawley rats (N = 6 for each age) were sacrificed under ether. Plasma [Na] and pH were measured by flame photometry and electrode, respectively. Compartmentation analysis was used to determine CP cell [Na], and pH (by the DMO method). SEM's were <8% of mean values below. [Na] and [H] are in mM and nM.

| | $[Na]_i$ | $[Na]_o$ | rNa | $[H]_i$ | $[H]_o$ | rH |
|---|---|---|---|---|---|---|
| 1 week | 58 | 137 | 0.42 | 63 | 45 | 1.40 |
| 2-3 wk | 46 | 148 | 0.31 | 65 | 40 | 1.63 |
| Adult | 47 | 154 | 0.31 | 87 | 36 | 2.42 |

The inwardly directed Na gradient (i.e., $[Na]_o - [Na]_i$) is nearly 30 mM less at 1 wk than in adults. At 1 wk, rH is only 3 times rNa whereas in young adults rH is 8 times rNa. Since Na-22 rapidly and completely exchanges with stable Na in rat CP, the concentration ratio of radio-Na (i.e., rNa) is likely similar to the activity ratio of stable Na. The substantially lower value of rH at 1 wk suggests a smaller proton-gradient driving force for uptake of Na into the cell. In a previous study in this laboratory systemic metabolic acidosis in adult rats reduced rH from 2.3 to 1.6, and it also decreased Na-22 turnover from plasma to CSF (across CP) by 35%. Since Na transport across CP is proportional to CSF formation, the present data are consistent with previous observations of lower-capacity Na transport in CP and incompletely-developed formation of CSF in the infant (1-wk) rat. Carbonic anhydrase activity, by catalyzing hydration of $CO_2$, furnishes H ions for the Na/H antiporter. Developmental increases in CP carbonic anhydrase activity, in addition to altered transmembrane gradients, undoubtedly contribute to the maturation of the CSF secretory process. Supported by NIH grant NS 13988.

246.2 PROSTAGLANDIN SYNTHESIS ($PGI_2$) IN CULTURED MICROVASCULAR CELLULAR ELEMENTS AND GLIA FROM RAT BRAIN: EFFECT OF ANGIOTENSINS AND BRADYKININ. B. Wroblewska*, O. Kempski*, N. Merkel*, J. Bembry* and M. Spatz. LNNS, NINCDS, National Institutes of Health, Bethesda, MD 20205.

The cellular prostaglandin interaction with angiotensin and kinins has been considered of great importance in the regulation of systemic blood pressure. The general association of prostaglandin synthesis with both peripheral and central vasculature is suggestive of a potential cerebrovascular prostaglandin-peptide interaction which might be involved in local regulation of the microcirculation. Therefore, we investigated the effect of angiotensin (I or II) or bradykinin on prostaglandin synthesis in separately cultured cerebrovascular endothelium and smooth muscle, and compared their responses to that in the glia.

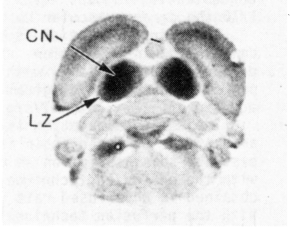
The propagated cultured endothelium and smooth muscle were derived from dissociated isolated cerebral microvessels while the cultured glial cells originated from dissociated cells of cortex. Washed cultures were exposed to medium G-199 with or without the tested substances (5μM) overnight. The synthesis of $PGI_2$ was measured indirectly in the medium of each cell type using radioimmunoassay with [$^{125}$I] 6-keto-$PGF_{1\alpha}$.

The greatest stimulation of prostaglandin release was observed in the medium of the smooth muscle cells exposed to either angiotensin (I or II) or to bradykinin. (The concentrations of 6-keto-$PGF_{1\alpha}$ were up to 100 times over the basal levels). The synthesis of prostaglandin in endothelium and glia was not significantly affected by the addition of angiotensin I. However, a slight enhancement of prostaglandin release from each cell line was observed after incubation with either angiotensin II or bradykinin (20-30% over basal level). Both, the lack and the low response of prostaglandin synthesis to the tested peptides in these cells in contrast to that of smooth muscle might be due to an enzymatic (kinase II) degradation of angiotensin I and bradykinin in the former but not in the latter cells.

These results strongly indicate that the cerebromicrovascular smooth muscle cells represent the most sensitive site for prostaglandin-peptide interaction which may be responsible for the modulation of vascular reactivity. The less responsive synthesis of prostaglandin to angiotensin and bradykinin observed in endothelium and glia suggests that these cells might serve as protectors of smooth muscle by inactivating peptides or by other mechanisms. Thus, each of the cells might have an influence on the cerebral microcirculation through its distinct and interrelated actions.

246.3 DIFFERENCES OF CAPILLARY DENSITY WITHIN THE INFERIOR COLLICULUS: RELATION TO LOCAL CYTOARCHITECTURE AND GLUCOSE METABOLISM. J.D. Fenstermacher, N.M. Sposito*, S.E. Nornes*, A.B. Butler and P.M. Gross, Department of Neurological Surgery, SUNY at Stony Brook, Stony Brook, NY 11794-8122.

The inferior colliculus (IC) of the midbrain tectum is composed principally of a large central nucleus (CN) of densely packed cells surrounded by a lateral zone (LZ) of fiber tracts (van Noort, The Structure and Connections of the Inferior Colliculus, Assen, van Gorcum, 1969). IC receives projections from the lateral lemnisci, cochlear nuclei, and auditory cortices, and is, therefore, a primary relay structure in acoustic processing. IC has the highest rate of glucose metabolism in the brain; moreover, within IC, this rate is greater in CN than in LZ (see Figure). A long-held hypothesis is that complexity of neuronal function, glucose metabolism, and capillary density are related throughout individual structures of the brain (Craigie, J. Comp. Neurol. 31:429, 1920). During auditory stimulation, glucose metabolism in the IC increases in patterns specific to tonotopic cellular organization within CN. We conducted quantitative light microscopic studies to discern a relationship of the capillary bed to the cellular organization and glucose metabolism within IC. Capillary mean diameter, density, and volume were determined from light micrographs of the IC prepared from perfusion-fixed brain sections of adult Sprague-Dawley rats. Glucose utilization was determined by computerized image processing of 14C-deoxyglucose autoradiographs from coronal brain sections of conscious rats.




Mean capillary diameter was similar in CN (4.61 ± 0.23 microns, mean ± SD) and LZ (4.54 ± 0.28 microns). Capillary density and volume, however, were greater in CN than in LZ. Values in CN were 564 ± 77 capillaries/$mm^2$ and 2.15 ± 0.28%, respectively and in LZ were 410 ± 73 capillaries/$mm^2$ and 1.39 ± 0.27%. Areas of high capillary density within CN correlated closely to groups of densely packed cells; LZ contained fewer capillaries, loosely distributed cell groups, and myelinated fibers. Glucose utilization was higher within CN. These studies illustrate the linear correspondence of cellular architecture, glucose metabolism, and capillary density and volume within an individual cerebral structure.

246.4 LOCAL SURFACE AREA AND QUANTITATIVE FINE STRUCTURE OF CAPILLARIES IN WHITE AND GREY MATTER AND A CIRCUMVENTRICULAR ORGAN OF RAT BRAIN. N.M. Sposito*, S.E. Nornes*, A.B. Butler, J.D.Fenstermacher and P.M. Gross (SPON: N.T. Carnevale). Department of Neurological Surgery, SUNY at Stony Brook, Stony Brook, NY 11794-8122.

From 1920 to 1940, E.H. Craigie published a series of reports describing the differences in capillary density among several structures of the rat brain having dissimilar functions. His studies led to the conclusion that "vascular richness must be associated with some special functional requirement" (Res. Publ. Assoc. Nerv. Ment. Dis. 20:310, 1940). We report a quantitative light and electron microscopic analysis of capillaries in individual brain structures having wide differences in neuronal function. The studies were carried out on adult Sprague-Dawley rats perfusion-fixed with 2% paraformaldehyde and 2% glutaraldehyde in 0.1 M phosphate buffer. Analyses were limited to the genu of the corpus callosum (white matter), inferior colliculus (IC, grey matter), and subfornical organ (SFO, a circumventricular organ, CVO). Values presented are the mean ± SD for the number of animals shown in parentheses. *$p<0.05$ compared to IC.

| Light Microscopy of Capillaries | Corpus Callosum | Inferior Colliculus | Subfornical Organ |
|---|---|---|---|
| Density (#/$mm^2$) | 150 ± 8(5)* | 500 ± 38(5) | 580 ± 62(3) |
| Volume (%) | 0.53 ± 0.04* | 1.76 ± 0.07 | 1.53 ± 0.14 |
| Surface area ($mm^2/mm^3$) | 4.1 ± 0.4* | 14.8 ± 1.2 | 15.2 ± 0.7 |
| Electron microscopy of endothelial cells | | | |
| Mitochondria (#/$um^2$) | 1.50 ± 0.11(3) | 1.38±0.34(3) | 0.49±0.11*(3) |
| Junctions (#$um^2$) | 0.46 ± 0.07 | 0.42 ± 0.07 | 0.67 ± 0.09* |
| Vesicles (#/$um^2$) | 2.6 ± 0.9 | 2.4 ± 0.3 | 16.7 ± 2.3* |
| Fenestrations (# / cross section) | none | none | 4.3 ± 0.8* |

The studies demonstrate quantitatively that white matter has fewer capillaries and less capillary volume and surface area than grey matter (IC) and SFO. The findings are consistent with the more energy demanding functions of grey matter and CVO structures. At the ultrastructural level, the SFO has fewer endothelial mitochondria and more intercellular junctions, vesicles, and fenestrations per unit cell area than white or grey matter. These values indicate morphological features which correlate with the lack of blood-brain barrier function in endothelial cells of CVOs.

246.5 A COMPARISON OF VASOPRESSIN BINDING TO PIG CEREBRAL MICROVESSELS, CORTEX, AND HIPPOCAMPUS. A.F. Pearlmutter* and S.I. Harik (SPON: A.V. McGrady), Department of Biochemistry, Medical College of Ohio, Toledo, OH 43699 and Departments of Neurology and Pharmacology, Case Western Reserve University School of Medicine, Cleveland, OH 44106.

Extensive anatomical evidence demonstrates the existence of nerve fibers in the walls of cerebral arteries and veins. Little is known of the influence of peptide neurotransmitters on the regulation of cerebral blood flow. Because injections of arginine vasopressin (AVP) have been shown to influence blood pressure, we tested cerebral microvessels for the presence of specific AVP binding sites. To clarify the nature of AVP binding to cerebral microvessels, we compared the microvessel system with pig cortical and hippocampal membranes, a brain area where we have previously located and characterized high-affinity, specific AVP binding in the rat (Costantini and Pearlmutter, J. Biol. Chem., 259:11739, 1984). Pig brains were collected at a local slaughterhouse and both microvessels and cortex were prepared as described previously (Harik et al., J. Cereb. Blood Flow Metabl, 1:329, 1981). Crude synaptosomal (12K) and membrane (100K) fractions of cortex and hippocampus were prepared by differential centrifugation as described previously (Pearlmutter et al., Peptides, 4:335, 1983). With the cerebral microvessels in 50 mM Tris, pH 7.6, little or no binding could be detected in the absence of metal ion or with 5 mM $Mg^{2+}$ or $Mn^{2+}$; similar results were obtained with pig cortex prepared in the same manner as the microvessels. When 5 mM $Ni^{2+}$ was included in the incubation medium, both microvessels and cortex bound AVP; the binding was of high affinity and saturable. By Scatchard analysis, the pig microvessels had a $K_d$ of 1.6 (±0.4) nM and a $B_{max}$ of 49 (±5) fmoles/mg protein; the cortex had a $K_d$ of 1.6 (±0.6) nM and a $B_{max}$ of 78 (±19) fmoles/mg protein. Although the differences in both $K_d$ and $B_{max}$ were not statistically different, within each set of parallel experiments, the cortex always exhibited about a 30% higher $B_{max}$ than the microvessels. Partially purified pig cortical and hippocampal membranes, both 12K and 100K, were tested for the presence of AVP binding sites. Both brain regions showed specific, high-affinity binding. In the pig cortex, the 12K fraction had a $K_d$ of 1.4 nM and $B_{max}$ of 113 fmoles/mg protein; the 100K membranes gave a $K_d$ of 1.3 nM and $B_{max}$ of 93 fmoles/mg protein. The pig hippocampal membranes also bound AVP; the 12K synaptosomal fraction had a $K_d$ of 1.6 nN and $B_{max}$ of 93 fmoles/mg protein while the 100K fraction had a $K_d$ of 0.9 nM and $B_{max}$ of 85 fmoles/mg protein. These results show that pig cortex and hippocampus bind AVP and that under conditions where specific AVP binding is detected in the cortex, pig microvessels can bind AVP. The binding conditions for AVP in the pig cerebral microvessels more closely resemble that seen previously with rat cortex and with pig cortex rather than a smooth muscle target tissue such as the aorta. This implies that AVP binding to microvessels resembles hormone binding seen in brain tissue. This research was sponsored by NIH grants NS-17848 to A.F.P. and NS-18150 to S.I.H.

246.6 THE BLOOD BRAIN BARRIER IS PERMANENTLY ALTERED BY TRANSPLANTS OF ADRENAL MEDULLA. J.M. Rosenstein. Dept. of Anatomy, George Washington Univ. Sch. of Med., Wash. D.C. 20037.

The adrenal medulla has been used in several neural transplant studies as a potential source of catecholamines. Like autonomic ganglia, the adrenal medulla normally lacks any blood-neuronal barrier. It has been recently shown that autonomic ganglion transplants circumvent the blood-brain barrier (BBB) and permit protein and amine to enter both the transplant and host brain (Rosenstein and Brightman, Science, 221:879). Might the adrenal medulla also subserve such a significant physiological mechanism following transplantation?

Adrenal medullae were dissected from young adult rat donors and transplanted to neonatal or adult recipients into either the IV ventricle or directly into the cerebral cortex. Postoperative times were between 4 weeks, to allow reconstitution of the BBB in the case of intraparenchymal trauma, to six months. A single injection of HRP was made into the femoral vein and allowed to circulate for periods between 3 minutes and one hour. Some animals received an injection of $^3$H dopamine ($^3$HDA) for comparable periods.

In all specimens, the circulating glycoprotein inundated the transplant and adjacent host brain. In IV ventricle transplants, several cerebellar folia were involved and HRP filled the breadth of the vermis to a depth of at least 1 mm. Use of the sensitive chromagen TMB demonstrated that HRP had transgressed the graft and entered and circulated in the CSF. Reaction product was detected rostrally in the cerebral aqueduct and caudally as far as the lumbar spinal cord; the paravascular spaces of the cerebral microvasculature were completely filled. In intraparenchymal allografts, months after the BBB should have been reconstituted, HRP was disseminated into the cortex, corpus callosum and hippocampus in as little as ten minutes. Serial reconstruction indicated that the volume of the permeable brain areas was 2-3 times the volume of the graft. Maximum HRP distribution was reached at 30 minutes indicating that the exudation was time dependent. In allografts that did not survive, no exudation was detected. Systemically administered $^3$HDA was found throughout the transplants and was sequestered in surviving cellular elements.

A fundamental characteristic of the mammalian brain, the BBB, is altered permanently and significantly by autonomic neural transplants by providing entry for circulating protein and amine. (Supported by NS-17468).

246.7 DIETHYLDITHIOCARBAMATE (DDC) CO-ADMINISTRATION ENHANCES LEAD-INDUCED FLAVOR-AVERSION CONDITIONING. D.B. Peele*, R.C. MacPhail*, J.D. Farmer* and L.L. Cook* (SPON:T.O. Brock). Northrop Services, Inc., Research Triangle Park, NC 27709 and U.S. Environmental Protection Agency, Research Triangle Park, NC 27711

Flavor-aversion conditioning represents an adaptive response involving food/fluid avoidance based on the consequences of prior ingestion of that substance. For example, rats treated with lead shortly after consuming saccharin-flavored water, and given a choice between the saccharin solution and tap water several days later, reduce their intake of saccharin in direct proportion to the dosage of lead administered (e.g., Peele, D.B. and MacPhail, R.C., Soc. Neurosci. Abstr., 10: 1207, 1984). The mechanism(s) by which lead administration produces this conditioned flavor aversion is(are) unknown. In an attempt to further characterize this phenomenon, a series of experiments was undertaken to determine whether the extent of flavor-aversion conditioning was related to lead levels in brain.

While only a small portion of orally delivered inorganic lead ordinarily reaches the brain, penetration of lead into brain can be greatly enhanced by co-administration of dithiocarbamates, including diethyldithiocarbamate (DDC), even though body burdens of lead remain roughly constant (Oskarsson, A., Arch. Toxicol., Suppl., 6: 279, 1984). If lead-induced flavor aversions are a function of brain lead levels, then the ability of lead administered in combination with DDC to induce flavor aversions should exceed that of lead administered alone. Experiment 1, therefore, established dosage-effect functions for flavor aversions induced by the sodium salt of DDC (0, 10, 30, 100, 300 mg base/kg, b.wt., i.p.). DDC produced dosage-related conditioned flavor aversions; a dosage of 10 mg/kg was determined to be without effect. In Experiment 2, flavor aversions induced by lead acetate (0, 30, 100 and 300 mg base/kg b.wt.) were assessed in rats jointly treated with either DDC (10 mg/kg) or isotonic saline. Lead produced dosage-related conditioned flavor aversions which were enhanced by the co-administration of DDC. A separate group of rats exposed to identical testing conditions was sacrificed 3 hrs after receiving lead (0 or 30 mg/kg) in combination with either DDC (10 mg/kg) or saline, and whole-brain lead levels were determined by atomic absorption spectrophotometry. The results showed an approximate 4-fold increase in brain lead levels in rats receiving lead plus DDC when compared to rats receiving lead plus saline. These results indicate that lead-induced conditioned flavor aversions may be, in part, determined by concentrations of the metal in brain.

(DBP was supported by an NRC Research Associateship.)

246.8 VALIDITY OF FOUR ASSUMPTIONS OF THE IN SITU BRAIN PERFUSION TECHNIQUE. Seiji Momma and Quentin R. Smith. Lab. of Neurosciences, NIA, NIH, Bethesda, MD 20205.

The in situ brain perfusion technique (Am. J. Physiol. 247: H484-H493, 1984) is a sensitive new method which we have used to measure amino acid transport across the blood-brain barrier. The principle advantage of this technique is that it allows absolute control of perfusate composition for the analysis of saturation and inhibition of cerebrovascular transport. However, there are four assumptions which must be verified before influx measurements for amino acids can be considered accurate. The objective of the following experiments was to evaluate the four assumptions.

The first assumption is that there is negligible mixing of perfusion fluid with circulating blood before the perfusate reaches the brain capillaries. Mixing is important because systemic blood contains amino acids which can competitively inhibit transport across the blood-brain barrier. The contribution of systemic blood to flow during perfusion was calculated from the brain uptake of $^{14}$C-iodoantipyrine during infusion of $^{14}$C-iodoantipyrine into the femoral vein and during brain perfusion with tracer-free fluid. The percent contribution of systemic blood equaled 0.23 ± 0.17% of total flow in the parietal cortex. This low level of mixing is insignificant because the maximal contribution of blood to perfusate amino acid concentration would be only 0.0023 x 0.9 = 0.0021 umol/ml, which is 1/12 the cerebrovascular Km for leucine.

The second assumption is that efflux of amino acids from brain does not contribute significantly to perfusate amino acid concentration in brain capillaries. This assumption is valid because the maximal contribution of efflux to mean capillary concentration is Ccap = Vmax/2F = 0.0027 umol/ml, which is only 1/10 the cerebrovascular Km for leucine.

The third assumption is that cerebral perfusion fluid flow is constant during perfusion. To verify this assumption we measured cerebral perfusate flow with $^{14}$C-diazepam after 3, 25, and 55 s of perfusion with physiological saline. In parietal cortex mean flow equaled 21 ± 2 x $10^{-2}$ ml/s/g and did not vary with perfusion time.

The fourth assumption is that the effective capillary surface area of the perfused brain is comparable to that in vivo. To evaluate this assumption we measured cerebrovascular PA to $^{14}$C-urea with the perfusion technique and compared the value to that obtained in unperfused rats with the i.v. injection technique. With the perfusion technique PA for $^{14}$C-urea equaled 7.0 ± 0.5 x $10^{-5}$ $s^{-1}$ and did not differ significantly from that in unperfused rats (6.6 ± 0.5 x $10^{-5}$ $s^{-1}$), which suggests that the capillary surface area during perfusion does not differ from that in vivo.

Thus, these findings indicate that the brain perfusion technique can be used to obtain accurate values for amino acid transport across the blood-brain barrier.

246.9 PRESENCE OF TRANSTHYRETIN mRNA IN RAT CHOROID PLEXUS: DEMONSTRATION BY IN SITU HYBRIDIZATION

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Transthyretin (TTR), formerly known as prealbumin, is a tetrameric protein composed of 4 identical polypeptide subunits of molecular weight 13,745. Plasma TTR is synthesized in the liver and functions in the transport of retinol and thyroxine. Recent studies have implicated a mutant TTR in the pathogenesis of familial amyloidotic polyneuropathy (type 1).

TTR forms a disproportionately large fraction of human ventricular protein, prompting the suggestion that it is either selectively transported across the blood-brain barrier or is synthesized de novo within the central nervous system (CNS). Immunocytochemical studies have demonstrated immunoreactive TTR within the cytoplasm of choroid plexus endothelial cells in humans and rats (Kato et al., submitted).

Recently we have isolated a cDNA clone from a lambda gt11 human liver expressing library, containing a 550 bp insert encoding an almost-full-length TTR sequence. Northern analysis employing nick-translated probe prepared from this insert identified a single hybridizing band of appropriate size (ca. 700 nt) in RNA extracts from rat brain (Soprano et al., submitted), and have demonstrated that the vast majority of this signal is derived from choroid plexus RNA. To investigate further the possibility of localized CNS TTR synthesis, we performed in-situ hybridization studies on rat brain sections using a $^{32}$P-nick-translated probe. The autoradiographs revealed an intense signal localized over the choroid plexus of the lateral, third, and fourth ventricles. Studies using tritiated probe to determine subcellular localization are in progress. These findings strongly support the suggestion (Soprano et al., submitted) that the choroid plexus is the site of specific de novo intra-axial TTR synthesis. Whether TTR serves a function in the the CNS different from that in the plasma is unknown at present.

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246.10 THREE-DIMENSIONAL RECONSTRUCTION OF ENDOTHELIAL VESICLES IN HUMAN AND ANIMAL GLIAL TUMORS. B.L.Coomber, P.A.Stewart, C.L.Farrell* and R.F.Del Maestro*. Dept. of Anatomy, Univ. of Toronto, Toronto, Ont., Canada M5S 1A8, and Brain Research Lab., Victoria Hospital, South Street Campus, London, Ont., Canada N6A 4G5.

Blood-brain barrier disruption in brain tumors results in cerebral edema, a major determinant of morbidity and mortality in the tumor patient. Current treatment for cerebral edema is limited to enhancing resorption of the edema fluid; we cannot yet prevent or even decrease the passage of fluids into the brain parenchyma. Animal models for human brain tumors, that could be used to investigate this problem, have been established; however, recent information indicates that the mechanism(s) of edema formation may be different in different tumor models. In the current study we have examined endothelial vesicles, that are thought to be involved in permeability by forming transcellular channels. Using three-dimensional reconstruction techniques, we compared vesicular arrangement in a rat glioma model with that in highly malignant glioblastoma multiforme tumors and low-grade astrocytomas in humans. Human tissue was obtained at biopsy and fixed within a few seconds of removal, so that optimal preservation of ultrastructure was obtained. Very thin (30-40 nm) serial sections were cut. Suitable areas of microvessel walls were photographed and vesicles were traced through adjacent micrographs. Vesicles were classified according to whether they are connected to other vesicles (fused), to golgi or ER (tubule connected), or unconnected to other cytoplasmic structures (free).

The frequency of each type of vesicle does not differ with tumor type, but is different from normal animal permeable tissue, in which the majority of vesicles are "fused", and often one or more vesicles form channels across the endothelial cell. In the current study, channels across tumor endothelial cells were rare. Unlike permable microvessels we have examined, the presence of fenestrated endothelium is variable in both human tumor vessels and rat glioma vessels. Our tumor endothelial cells were also not like normal animal barrier endothelium, in that the majority of vesicles in barrier endothelium are "tubule connected", and transendothelial channels and fenestrations are never seen. From the above results we conclude that, with regard to vesicular arrangement, tumor microvessels are not like normal brain barrier vessels, nor are they like normal permeable vessels. Therefore, the mechanism(s) of endothelial permeability in brain tumor vessels is unlikely to be same as that in normal permeable vessels.

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246.11 LACK OF CORRESPONDENCE BETWEEN INCREASED PROTEIN PERMEABILITY AND NEURONAL ACTIVATION OR DEATH FOLLOWING HARMALINE-INDUCED TREMOR. R. E. Ruth, Inst. Study Dev. Disabil., Univ. Illinois, Chicago, IL 60608.

The β-carboline, harmaline, induces rhythmic tremor caused by increased bursting of inferior olivary (io) neurons. Concomitantly discharges appear in the cerebellum and those brainstem areas receiving cerebellar efferents. Surgical isolation experiments localize the action of harmaline to the io itself. Despite this widespread electrical activation only certain parts of io and the red nucleus manifest increased glucose metabolism (Bardin et al., 1983).

To observe if cerebrovascular protein permeability (CVP-p) increased during such tremors we injected freely moving rats with harmaline (20mg/kg, ip) followed either 2 or 60 minutes later with horseradish peroxidase (HRP, 35mg, iv). One hour after HRP injection rats were anesthetized and perfused. Brain sections were processed for the blue HRP reaction product. Control rats received harmaline alone or HRP alone to assess endogenous peroxidase activity and the normal pattern of CVP-p, respectively. A final group of rats was injected with harmaline and survived two days for neuropathologic examination of io using the Fink-Heimer method.

Under normal conditions HRP was found only in circumventricular organs; no endogenous peroxidase activity was evident. In harmaline/HRP-injected cases profuse reaction product was localized within io. In the cerebellum infrequent, discrete "spots" of reaction product appeared in the vermal molecular layer but nowhere else. No reaction product was observed in other brainstem areas activated electrically by harmaline nor in the red nucleus. Neuropathologic examination indicated no pyknotic or argyrophilic neurons within io, despite tremors lasting at least 4 hours in these cases.

The results indicate only a weak relationship between the anatomical localization of increased CVP-p and increased glucose metabolism. In addition the increased CVP-p within io is not associated with any long-term adverse effects on io neurons.

247.1 CHARACTERIZATION OF DIFFERENCES IN THE PHOSPHORYL RESIDUE OF NON-AGED AND AGED ORGANOPHOSPHORYL CONJUGATES OF SERINE HYDROLASES: $^{31}$P-NMR SPECTROSCOPY OF PHOSPHORYLATED α-CHYMOTRYPSIN. J. Grunwald*[1], Y.Ashani*[1], Y. Segall*[1], D. Waysbort*[1], N. Steinberg*[2] and I.Silman*[2] (SPON: M.Spiegelstein) Israel Institute for Biological Research, P.O.Box 19, Ness Ziona, Israel[1] and The Weizmann Institute of Science, Rehovot, Israel[2].

The powerful acute toxicity and delayed neurotoxicity of organophosphorus (OP) esters is attributed primarily to their irreversible inhibition of serine hydrolases such as AChE and neurotoxic esterases. In several cases, the inhibited enzyme undergoes a process, commonly termed aging, where the inhibited enzyme can no longer be reactivated. In order to provide basic information at the molecular level which may explain the unexpected stability of aged OP-enzyme conjugates, pyrenebutyl-O-P(O)($OC_2H_5$)F(PBEPF) and pyrenebutyl-O-P(O)($OC_2H_5$)Cl(PBEPC) were used to obtain the non-aged form [pyrenebutyl-O-P(O)($OC_2H_5$)Cht] where as pyrenebutyl-O-P(O)$Cl_2$(PBPDC) and pyrenebutyl-OP(O)(O$\Phi NO_2$)Cl[PBP(pNP)C] were used to produce the aged conjugate pyrenebutyl-O-P(O)(OH)Cht of α-chymotrypsin (Cht). The availability of highly purified large quantities of Cht enabled to prepare solutions of OP-Cht conjugates in the millimolar range, allowing not only fluorescence spectroscopy but also $^{31}$P-nmr spectroscopy. For the conjugates obtained from PBEPF, PBEPC, PBPDC and PBP(pNP)C the ratio of absorption at 344 and 280 nm indicated an approximately 1:1 (±15%) stoichiometry of OP and protein in the conjugate.The relative chemical shifts of the phosphorylated non-aged and aged Cht were found to be as predicted from the corresponding $^{31}$P-nmr chemical shifts of model compounds where the $^{31}$P-nmr signals of the non-aged form appeared ~1 ppm and ~1.7 ppm upfield to the aged conjugate for the native and denatured preparations respectively. The non-aged form could be reactivated in the presence of 3-pyridinealdoxime methiodide (3-PAM) whereas no reativation was observed for the similarly treated aged conjugate. The $^{31}$P-nmr results and the reactivation data strongly support the hypothesis that aged form of OP-Cht conjugate contains P-O$^{(-)}$ bond. These results, along with the fluorescence spectroscopy data (see following abstract) may provide partial interpretation for the known resistance of the aged conjugates of serine hydrolases to reactivation.

247.2 CHARACTERIZATION OF DIFFERENCES IN THE ACTIVE-SITE CONFORMATION OF NON-AGED AND AGED ORGANOPHOSPHORYL CONJUGATES OF SERINE HYDROLASES: OPTICAL SPECTROSCOPY OF PHOSPHORYLATED α-CHYMOTRYPSIN. N. Steinberg*[1], A.C.M.Van der Drift*[1], E.Haas*[1], I.Silman*[1],J.Grunwald*[2] and Y.Ashani*[2] (SPON:N.Allon). The Weizmann Institute of Science, Rehovot[1], and Israel Institute for Biological Research, P.O.Box 19,Ness Ziona[2],Israel

Two kinds of stoichiometric conjugates formed between α-chymotrypsin (Cht) and fluorescent organophosphorus (OP) cpds were examined by optical spectroscopy in order to probe their active sites. The non-reactivatable (aged) pyrenebutyl-OP(O)(OH)Cht was prepared by the reaction of either pyrenebutyl-OP(O)$Cl_2$ or pyrenebutyl-O-P(O)(O$\Phi NO_2$)Cl with Cht, whereas the reactivatable (non-aged) conjugate pyrenebutyl-OP(O)-($OC_2H_5$)Cht was prepared by reacting pyrenebutyl-OP(O)-($OC_2H_5$)F with Cht (see preceding abstract). The circular dichroism (CD) spectra of pyrenebutyl-OP(O)-($OC_2H_5$)Cht and of pyrenebutyl-OP(O)(OH)Cht in the tryptophans' absorption region is similar. However, the absorption anisotropy factor ($g_{ab}$) in the pyrene absorption region (ca. 350nm) is markedly more negative for the non-aged than for the aged form of OP-Cht conjugate. Similar results were obtained from circularly polarized luminescence (CPL) measurements. The emission anistropy factor of the aged conjugate obtained by two different inhibitors is the same within experimental error, and is different in both magnitude and sign from that of the non-aged conjugate. Steady-state fluorescence measurements of the conjugates reveal a similar fluorescence profile; the quantum yield of pyrenebutyl-OP(O)(OH)Cht is ca. 20% lower than that of pyrenebutyl-OP(O)($OC_2H_5$)Cht. Supporting evidence was obtained from fluorescence lifetime measurements. The fluorescence decay data of all the conjugates could be described by a two exponent decay function. The short component, ca. 10 nsec, was found to be equal for the aged and the non-aged OP-Cht conjugate, while the long component assumed the values of ca. 88 nsec and 98 nsec respectively. The data obtained by optical-spectroscopic methods clearly indicates different active-site conformations for the aged and non-aged conjugates. It may further be concluded that the position of the pyrene fluorophore relative to the polypeptide chain is different in the two conformations. These differences may be assigned to the different substituents attached to the P-atom.

247.3 VOLTAGE-DEPENDENT SLOW MEMBRANE CURRENTS IN RAT SPINAL DORSAL HORN NEURONS AND THE ACTIONS OF SUBSTANCE P. K. Murase and M. Randić (SPON: J. R. Carithers). Information and Computer Science, Toyohashi Univ. of Technology, Tempaku, Toyohashi, 440 Japan, and Dept. of Vet. Physiol. and Pharmacol., Iowa State Univ., Ames, IA 50011.

Rat (10-26 days old) spinal dorsal horn neurons in vitro were voltage-clamped by means of a single microelectrode sample-and-hold technique. In 22 out of 38 neurons examined, hyperpolarizing voltage commands from relatively positive holding potentials (-36 to -56 mV) elicited a slow inward current relaxation with voltage-dependent properties similar to the M-current, first described in amphibian sympathetic ganglion cells (Brown and Adams, Nature, 283:673-676, 1980). Increasing the bathing medium potassium concentration shifted the reversal potential of the curent relaxation to a less negative potential as predicted for a potassium current. The current is depressed by external $Ba^{2+}$ ions (0.5-4.0 mM), and in a few cells by muscarine (10-100 μM), but not by external $Cs^+$ (1 mM). In contrast, in 24 neurons, slow inward relaxations triggered by steps negative to -70 mV appeared to reflect another current resembling an anomalous rectifier, $I_Q$, of CA1 hippocampal neurons. This current is blocked by external $Cs^+$, but is not sensitive to muscarinic agonists or $Ba^{2+}$ ions. Substance P (SP, $10^{-7}$-$10^{-5}$M, Cambridge Research Biochemicals) reduced the M-like current in dorsal horn neurons irrespective of their muscarinic sensitivity. In addition, SP induced a novel inward relaxations in several cells clamped at a holding potential of -60 mV and subjected to one-second depolarizing voltage jumps. It is concluded that the coexistence of both SP effects in rat dorsal horn neurons might account for the SP-induced depolarization associated with a small conductance change in these cells.

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247.4 GANGLIONIC BLOCKADE BY CLONIDINE. D. Christ. South Bend Center for Medical Education, Indiana University School of Medicine, Notre Dame, IN 46556.

Clonidine is an alpha-adrenoceptor agonist with antihypertensive actions. As catecholamines block synaptic transmission in autonomic ganglia by acting at alpha-adrenoceptors, an investigation was initiated to determine whether clonidine had effects on ganglia like the catecholamines. Compound action potentials were recorded from the postganglionic nerves of hamster isolated stellate ganglia that were superfused with an oxygenated, warmed physiological solution. When clonidine was added to the superfusing solution, the amplitude of the compound action potential was reduced. The onset of blockade was rapid, but full recovery usually had not occurred after 30 min in the control solution. Blockade was observed at concentrations of clonidine, as low as $10^{-8}$ M; but $10^{-4}$ M was necessary for complete blockade of the compound action potential. The slope of the concentration-blockade relationship for clonidine was not as steep as the slope of the concentration-blockade relationship for norepinephrine. The blockade at the lower concentrations of clonidine was reduced by yohimbine ($10^{-6}$M), an $alpha_2$-adrenoceptor antagonist; but yohimbine had little effect on the blockade by the higher concentrations of clonidine. In the presence of clonidine, paired preganglionic stimulation resulted in a marked facilitation of the second compound action potential if the stimulus interval was less than 200 ms. The effect of clonidine on ganglionic afterdischarges was also observed. The preganglionic nerve was stimulated at a frequency of 30 Hz for 2 s in the presence of hexamethonium ($10^{-3}$ M) to induce the asynchronous afterdischarges that appear to be mediated by muscarinic cholinergic mechanisms. Clonidine reduced the afterdischarges at concentrations greater than $10^{-9}$ M. $10^{-7}$ M Clonidine produced nearly 100% blockade, thus the afterdischarges were more sensitive to the blocking action of clonidine than the compound action potential. Yohimbine ($10^{-6}$ M) also reduced this action of clonidine. These results indicate that clonidine is very effective in blocking ganglionic transmission that is mediated by nicotinic or muscarinic mechanisms. The effect of clonidine appears to involve an action at $alpha_2$-adrenoceptors in the ganglion.

247.5 ACTIONS OF PHYSOSTIGMINE AND PYRIDOSTIGMINE ON CAT SPINAL CORD RENSHAW CELLS. W.G. VanMeter, K.C. Sikora-VanMeter, and R.C. Wierwille*. USAMRICD, Aberdeen Proving Ground, MD 21010-5425; Dept. of Veterinary Physiology and Pharmacology, Iowa State Univ. Ames, IA 50011, and Dept. of Pharmacology, School of Medicine, University of Maryland, Baltimore, MD 21201.

Responses of a cholinergic transmitting CNS synapse (spinal motoneurone axon collateral-Renshaw cell interneurone) to a tertiary carbamate (physostigmine) and to a quaternary carbamate (pyridostigmine) were compared in adult male cats of mixed breed. After DIAL anesthesia (80 mg/Kg, ip.), laminectomies were performed (S-1 to T-13), the spinal cord transected (T-13) and the spinal roots (dorsal and ventral) from segments L-5 to S-1 were separated, cut and placed on platinum stimulating electrodes. Extracellular unit potentials evoked by antidromic stimulation of the ventral roots were recorded by conventional methods from glass micropipettes (2.7M NaCl;1.0-1.5 micron tips) positioned in the ipsilateral spinal segments (usually L-7). Physostigmine salicylate (MW 413.5) induces a dose-dependent increase in frequencies and a marked increase in duration of the burst response. The latter increases in duration from 50-70 msec during predrug controls to a burst response in excess of 1 second after intravenous administration of the anticholinesterase (0.1-0.5 mg/Kg). Similarly, pyridostigmine bromide (MW 261) alters the burst frequencies and durations but to a much lesser extent (50-70 msec during predrug controls to 100-150 msec). Moreover, the dose range required is of greater magnitude (0.5-5.0 mg/Kg i.v.). Both anticholinesterase-induced responses are insensitive to atropine methyl nitrate (0.3 mg/Kg iv.) and to atropine sulfate (0.5-1.0 mg/Kg iv.) but are antagonized by mecamylamine HCl in a dose dependent manner (0.2-2.0 mg/Kg iv.). This antagonism is dose cummulative, persistent and not reversed by the subsequent administration of either anticholinesterase. An additional series of experiments in which drug treatments were carried out in vivo followed by in vitro histochemical verification of cholinesterase activities, shows pretreatment with either carbamate (physostigmine (0.2 mg/Kg iv.) or pyridostigmine (2.5 mg/Kg iv.)) to be effective in protecting the enzyme from irreversible inhibition by the subsequent administration of soman (0.01 mg/Kg iv.), with the stain being more pronounced with the tertiary carbamate. These data suggest pyridostigmine gains access to the CNS if given in sufficiently high doses and is consistent with a CNS action which heretofore has been inferred from indirect observations (Wolthuis, O.L. and Vanwersch, R.A.P. (1984) Fundam. Appl. Toxicol. 4: S195).

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247.6 EFFECTIVENESS OF PRETREATMENT WITH PHYSOSTIGMINE (PHY) AND MECAMYLAMINE (MEC) AGAINST LETHAL EFFECTS OF IRREVERSIBLE ORGANOPHOSPHORUS (OP) CHOLINESTERASE (ChE) INHIBITORS IN RATS. S.S. Deshpande*, G.B. Viana*, M. Kawabuchi*, A.F. Boyne, D.L. Rickett and E.X. Albuquerque. (SPON: D.R. Burt). Dept. Pharm. & Exp. Ther., Univ. of Maryland School of Medicine, Baltimore, MD 21201.

The reversible ChE inhibitor PHY can protect rats against a lethal dose ($LD_{100}$, 0.13 mg/kg) of sarin (Meshul et al., Neurosci. Abs., 1984). Female Wistar rats (220 g) were injected (i.m.) with PHY (0.1 mg/kg) and atropine (ATR, 0.5 mg/kg) or PHY + ATR and a ganglionic blocking drug MEC (4 mg/kg) or chlorisondamine (2 mg/kg). After 30 min, sarin (0.65 mg/kg, equivalent of 5 x $LD_{100}$ dose) or VX (0.05 mg/kg, equivalent of 4 x $LD_{100}$ dose) was injected subcutaneously. Blood ChE and muscle and brain acetycholinesterase (AChE) were inhibited by 50, 42 and 62% respectively (vs. 88, 82 and 98% in rats receiving sarin alone) in pretreated animals receiving sarin. Pretreatment with PHY + ATR produced 100 and 50% lethality after sarin and VX respectively. PHY + ATR + (MEC or chlorisondamine) reduced VX lethality from 50 to 0%. VX caused a 4-fold potentiation of single twitches (in vivo) of the extensor digitorum longus muscle within 5 min of injection, and the muscle was unable to maintain tension during 2 sec nerve stimulation at 50 Hz. These rats died at 15 min. A 2-fold potentiation of single twitch without loss of ability to sustain tetanus (50 Hz) was seen 30 min after PHY + ATR. An injection of VX (0.05 mg/kg) in these rats produced further twitch potentiation accompanied by failure to maintain tetanic tension and post tetanic depression of single twitches. Partial (at 6 hr) and complete recovery (at 24 hr) of muscle contraction were observed in rats receiving VX after PHY + ATR. In vitro recordings from endplate regions of diaphragm or soleus muscles showed an 8-10 fold increase in miniature endplate potential (MEPP) frequency without any alteration in membrane potential with 0.1 μM VX perfusion. Soleus muscles from rats receiving VX after pretreatment with PHY + ATR showed ~6 mV membrane depolarization at 24 hr. Electronmicroscopic examination of soleus muscles at 24 hr after injection of VX alone (0.005 mg/kg, sublethal dose) disclosed extensive subsynaptic vacuolization of mitochondria, disruption of sarcoplasmic reticulum and subjunctional myofibrillar architecture. In conclusion, PHY + ATR and PHY + ATR + MEC pretreatments were effective in protecting rats from the lethal effects of VX. Inability of PHY + ATR to protect rats from 5 x $LD_{100}$ dose of sarin argues against protection by carbamates of a critical pool of AChE from phosphorylation by OP agents. The mechanism of protection by PHY and MEC against sarin and VX should take into account the direct actions of these agents, PHY & MEC on the nicotinic receptor-ion channel located in the peripheral and central nervous system (Supported by U.S. Army Grant DAMD-17-84-C-4219).

247.7 EFFECT OF MUSCARINIC STIMULATION ON CALCIUM MOBILIZATION, PHOSPHOINOSITIDE METABOLISM AND TRANSMITTER RELEASE FROM PC12 CELLS. Carolyn S. Rabe and Forrest F. Weight Lab of Preclinical Studies, Nat'l Institute on Alcohol Abuse & Alcoholism, Rockville, MD 20852.

The rat pheochromocytoma cell line, PC12, has many characteristics in common with sympathetic neurons. In addition to nicotinic receptors, whose activation stimulates secretion, muscarinic binding sites have been identified on PC12 cells (Nature 297: 152, 1982). However, little is known regarding the functional significance of these muscarinic binding sites. Recently we have studied the effect of muscarinic agonists on intracellular $Ca^{2+}$ mobilization, phosphoinositide metabolism and transmitter release from PC12 cells. Intracellular free $Ca^{2+}$ was measured using the fluorescent $Ca^{2+}$ indicator Quin2. Cells were loaded with 10 uM Quin2 AM for 20 min, washed and resuspended at a concentration of 2 x $10^6$ cells/ml in HEPES buffered saline containing 1.8 mM $Ca^{2+}$. The cells had a resting free intracellular $Ca^{2+}$ level of approximately 110 nM using the calibration method of Tsien et al. (Nature 295: 68, 1982). Addition of muscarinic agonists of the low affinity subtype, $M_2$, methacholine (100 uM) or muscarine (100 uM), caused a 50-60 nM increase in intracellular free $Ca^{2+}$ levels. In contrast, agonists of the $M_1$ subtype, oxotremorine (1 mM) or McN-A-343-11 (1 mM), had no detectable effect on intracellular $Ca^{2+}$ levels. The increase in intracellular $Ca^{2+}$ produced by methacholine was blocked by atropine (100 nM). However, removal of $Ca^{2+}$ from the extracellular medium did not block the ability of the $M_2$ agonists to raise intracellular $Ca^{2+}$ levels. This suggested that the increase in intracellular $Ca^{2+}$ might be the result of mobilization of $Ca^{2+}$ from intracellular stores. Consistent with that hypothesis was the observation that methacholine treatment increased cellular levels of inositol triphosphate ($IP_3$), a metabolite of phosphatidylinositol bisphosphate shown to mobilize $Ca^{2+}$ from intracellular stores in several other cell types (Nature 312: 315, 1984). When cells had been prelabeled with [$^3$H]inositol (5 uCi/ml) for 24 hr, methacholine (200 uM) doubled [$^3$H]$IP_3$ levels within 30 sec (control 993 ± 71; methacholine 1960 ± 15 cpm/mg protein). The rate at which [$^3$H]$IP_3$ levels accumulated slightly preceeded the rate of increase in the Quin2 $Ca^{2+}$ signal. The levels of [$^3$H]inositol bisphosphate and [$^3$H]-inositol phosphate also increased, but with a slightly slower time course. We have also begun to examine the effects of $M_2$ receptor activation on secretion. In the absence of other stimuli, methacholine (100 uM) caused a small stimulation of catecholamine release from PC12 cells which had been preloaded for 30 min with [$^3$H]norepinephrine (500 nM, 23.1 Ci/mmol). Although it is unclear at this time whether the $M_2$ activated secretion was a direct result of $Ca^{2+}$ mobilization, the PC12 cells clearly provide a model system for examining the interrelationship between $M_2$-induced phosphatidylinositol metabolism, intracellular free $Ca^{2+}$ and secretion.

247.8 CHOLINERGIC FUNCTION IN MOUSE SPINAL CORD CELL CULTURES. F.Z.Wang* and P.G. Nelson. Lab. of Developmental Neurobiology, NICHD, NIH, Bethesda MD 20205.

Cholinergic synapses evoke both rapid, transient (nicotinic) and slow, sustained (muscarinic) excitation of mammalian central neurons. Slow, sustained depolarizing responses evoked by cholinergic agonists and reversibly antagonized by atropine were present in cultured spinal cord neurons (Linda M. Nowak & Robert L. Macdonald. J. Neurophysiology 49:792-803, 1983). We investigated cholinergic synaptic responses and responses to cholinergic agonists in mouse spinal cord neurons in cell culture to determine the mechanisms of cholinergic synaptic transmission between mammalian central neurons.

Intracellular recordings were made from dissociated fetal mouse spinal cord neurons and dorsal root ganglion (DRG) cells greater than 4 weeks in culture; in some cases rat superior cervical ganglion (SCG) neurons were added to the spinal cord (SC) cells. Experiments were performed in the presence of 5 mM $Mg^{++}$ and 5 mM $Ca^{++}$ using 3M KAc filled microelectrodes. Cholinergic SC neurons could be identified by the fast EPSP which they elicited in the SCG neurons. Pressure applications of acetylcholine (ACh), muscarine (MUS) or atropine from drug-filled perfusion pipettes were used to assess cholinergic effects on neuronal excitability or synaptic transmission between SC neurons or between DRG and SC neurons. In confirmation of others (Macdonald et al.), ACh or MUS produced an increase in the excitability of more than 3/4 of SC neurons tested; this was associated with an increase in neuronal membrane resistance. In 2 DRG neurons, hyperpolarizing responses were obtained. Excitatory synaptic connections tested with ACh or MUS were augmented while 2 IPSP were substantially diminished. The pre- or post-synaptic locus of these effects have yet to be determined. Stimulation of a SC neuron identified as cholinergic by its innervation of SCG cells could produce a prolonged, recurrent, depolarizing excitation of the cholinergic neuron itself which was blocked with atropine. Relatively rapid, depolarizing synaptic potentials in adjacent SC neurons were elicited by a cholinergic SC neuron, and in 'cholinoreceptive' neurons, ACh produced relatively rapid depolarization.

247.9 TWO RECEPTOR-IONOPHORE COMPLEXES MEDIATE THE ACTION OF GABA IN THE GUINEA-PIG HIPPOCAMPUS. N. Ogata*, M. Inoue*, and T. Matsuo* (SPON: C.H. Wu). Dept. of Pharmacol., Fac. of Med., Kyushu Univ., Fukuoka 812, Japan.

The ionic mechanism underlying the action of γ-aminobutyric acid (GABA) in the hippocampus was studied using brain slices. GABA, either perfused or ionophoresed, produced a triphasic membrane potential change in the pyramidal cells, comprising an initial fast hyperpolarization, a sustained depolarization, and a late hyperpolarization. When GABA was applied at low concentrations, the depolarizing component of the triphasic GABA response was selectively dissipated and the two hyperpolarizing components were fused to a monophasic hyperpolarization. The depolarizing component was augmented by a reduction of external $Na^+$.

The GABA-induced depolarization was markedly augmented by the substitution of external $Cl^-$ with an impermeant anion and was depressed by a low dose of picrotoxin ($10^{-5}$ M). Combined application of picrotoxin and a low concentration of bicuculline ($10^{-5}$ M) eliminated the major part of the depolarizing component in the triphasic GABA response and thus the two hyperpolarizations were fused to a monophasic hyperpolarization. Baclofen, a $GABA_B$ agonist, produces a prominent hyperpolarization due to an activation of $K^+$ conductance in the hippocampus (Inoue et al., Brit. J. Pharmacol., in press). The reversal point of the GABA-induced hyperpolarization approximated that of the baclofen-induced hyperpolarization. In addition, the GABA-induced hyperpolarization was augmented in low $K^+$ medium and reduced in high $K^+$ media but unaffected by a change in external $Cl^-$.

The hyperpolarization induced by baclofen but not by GABA showed prominent voltage dependence. The membrane depolarization markedly attenuated the response to baclofen while the decrease in input resistance was progressively enhanced by hyperpolarizing the membrane. The action of baclofen was strongly antagonized by a low dose ($5x10^{-6}$M) of 4-aminopyridine, whereas that of GABA was insensitive to a very high dose ($10^{-3}$M) of this drug. The slow inhibitory postsynaptic potential (slow i.p.s.p.) evoked by orthodromic stimulation was readily suppressed by a low dose of baclofen but not by GABA.

The results indicate that the GABA response in the hippocampus is mediated by at least two independent receptor-ionophore complexes. One is low-affinity receptors linked to $Cl^-$ channels and responsible for the depolarizing action of GABA, and the other is high-affinity receptors linked to $K^+$ channels and responsible for the hyperpolarization. The initial and late hyperpolarizations induced by GABA may result from activation of a common $K^+$ conductance distinct from the $K^+$ conductance responsible for the hyperpolarizing action of baclofen. The slow i.p.s.p. does not appear to be mediated by GABA, while baclofen may act as a potent agonist for receptors mediating the slow i.p.s.p.

247.10 RESPONSES TO GABA ON THE CELL BODIES AND DENDRITES OF RAT VISUAL CORTICAL NEURONS IN TISSUE SLICES. H. E. Scharfman and J. M. Sarvey. Dept. Pharm., Uniformed Services Univ. of the Health Sciences., Bethesda, MD 20814.

There is substantial evidence that GABA may be the transmitter of many inhibitory neurons in the visual cortex. To investigate the effects of GABA on visual cortical cells, intracellular recordings were obtained from tissue slices (450um thick) of rat visual cortex.

Recordings were obtained from 80 neurons located in layers II-VI (RMP=-65 ± 1.3mV; action potential amplitude=79 ± 1.2mV; $R_{in}$=31 ± 2.8mohms; mean ± sem). Ten cells were stained by intracellular injection of the fluorescent dye lucifer yellow and were identified as both pyramidal and nonpyramidal.

32 of 33 cells responded to pressure application (10-35psi, 5-1000msec pulses) of GABA (10mM) to the soma or dendrites. GABA responses were brief, lasting only seconds, and were associated with a conductance increase. Responses were composed of two components, the first with a reversal potential of -65.5 ± 0.5 mV (n=7), and the second with a reversal potential of -54mV ± 0.9mV (n=4). The first was hyperpolarizing or depolarizing, depending on the RMP, and observed following somatic or proximal dendritic GABA applications. When recording electrodes were filled with 3M KCl, the responses at the soma were only depolarizing, and had a more positive reversal potential (-48mV, n=2). Therefore, it is likely that chloride ions are involved in this response. The second type of GABA response was always depolarizing, and could be recorded after GABA application to any area of the cell.

Subthreshold and suprathreshold responses to synaptic stimulation or intracellular current injection were partially or completely blocked during GABA responses, which supports the hypothesis that GABA has an inhibitory action.

Addition of the GABA receptor antagonist bicuculline methiodide (50uM, n=3) to the perfusing medium blocked GABA responses which reversed near -54mV, but had little or no effect on the responses which reversed at -65mV.

It appears that almost all visual cortical cells respond to GABA applied on their cell bodies and dendrites, and are affected in a similar manner. The effects of GABA are also similar to those of hippocampal pyramidal cells. These studies provide some insight into the inhibitory influences on cortical activity.

247.11 PHENCYCLIDINE (PCP), A NEW POTENT ANTAGONIST FOR GLUTAMATE RECEPTOR. M. Idriss* and E.X. Albuquerque. (SPON: A.T. Eldefrawi). Dept. Pharmacol. & Exp. Therap., Univ. Maryland, School of Medicine, Baltimore, MD 21201.

The general anesthetic and hallucinogenic agent "Angel Dust" [1-(1-phenylcyclohexyl)piperidine; phencyclidine; PCP] is a drug of abuse which, when used chronically or in large doses, has schizophrenomimetic and convulsant properties and triggers unpredictable and dangerous violent behavior. A large number of studies have been devoted to PCP, and it has been demonstrated that this agent is able to affect the release and to interact with the recognition sites of many neurotransmitters. Albuquerque and coworkers initially showed that this agent blocks the voltage-sensitive K channels in the nerve and muscles thus resulting in prolongation of the action potential which can be responsible for increase in transmitter release from nerve terminals in many synapses. Further, the site of action of PCP as a noncompetitive blocker of the nicotinic acetylcholine (ACh) receptor-ionic channel complex involves an increase in affinity of ACh for its recognition site, subsequent desensitization and open channel blockade. Recently we have observed that PCP also interacts with the glutamatergic synapse. Such an action is reported here.

PCP at concentrations between 5 and 40 μM was tested on metathoracic tibialis muscles of Locusta migratoria. The drug produced significant voltage-dependent depression of endplate current (EPC) peak amplitude and accelerated the EPC decay time constant ( $\tau_{EPC}$ ). The depression of EPC peak amplitude induced by PCP was more prominent at hyperpolarized membrane potentials and was coupled with nonlinearity in the current-voltage relationship. PCP caused a significant shortening of $\tau_{EPC}$, and an increase in the drug concentration produced an apparent loss of the voltage-sensitivity recorded under control conditions. At -80 mV holding potential, PCP (5, 20 or 40 μM) depressed the EPC peak amplitude by 10, 50 and 67% and reduced $\tau_{EPC}$ by 28, 50 and 60% respectively. Fluctuation analysis indicated that PCP shortens channel lifetime but does not affect channel conductance. Also the alterations of the EPC characteristics induced by PCP involved a shortening of the EPC rise time.

The present study clearly demonstrates that PCP, at concentrations similar to those for blockade of the neuromuscular transmission, blocks the glutamate receptor ion channel in open conformation according to a mechanism similar to that of the nicotinic synapses. (Supported by U.S. Army Res. & Devel. Command Contract DAMD-17-84-C-4219.)

247.12 INTERACTIONS OF PHENCYCLIDINE (PCP) AND ITS DERIVATIVES WITH THE ACETYLCHOLINE-ACTIVATED ION CHANNEL. L.G. Aguayo* and E.X. Albuquerque. (SPON: G.J. Markelonis, Jr.). Dept. Pharmacol. & Exp. Therap., Univ. Maryland Sch. of Med., Baltimore, MD 21201.

Interactions of the hallucinogenic drug PCP (1-(1-phenylcyclohexyl) piperidine) and some of its analogs with the nicotinic ACh receptor-ionic channel complex were studied using electrophysiological techniques. The peak amplitude and the decay time constant of the nerve-evoked endplate currents (EPCs) recorded from the frog sartorius muscle were reduced by all the analogs in a concentration-dependent manner ($IC_{50}$ between 5 and 90 μM). PCP, TCP (1-[1-(2-thienyl)cyclohexyl] piperidine), and PCE (N-ethyl-1-phenylcyclohexylamine), among other analogs caused a negative slope conductance in the current-voltage relationship and a voltage- and time- dependent blockade of the peak amplitude of the EPC. When the piperidine ring of PCP was substituted by a morpholino ring, as in PCM (1-(1-phenylcyclohexyl) morpholine) and TCM (1-[1-(2-thienyl)cyclohexyl]morpholine), the potency decreased and the negative conductance was eliminated. The removal of the piperidine ring in PCA (1-phenylcyclohexylamine) and the hydroxylation of the cyclohexane ring in PPC (4-phenyl-4-piperidino-cyclohexanol) reduced the potency and produced double exponential decays at potentials between +50 and -50 mV. At -100 mV, the potency for EPC blockade was well correlated with the potency for reducing the decay time constant for all the analogs. The behaviorally active analogs were the most potent EPC blockers, which suggests a synaptic role for the production of behavioral alterations. TCP (5-25 μM) produced a large reduction in the peak amplitude without affecting its decay time constant (dynamic blockade). This blockade was independent of receptor activation and was reversed at positive potentials (i.e. +50 mV). Single channel currents in the presence of PCP (4 μM) or PCM (10 μM) revealed a decrease in channel openings and mean channel lifetime without any change in single channel conductance.

These results suggest that PCP and its analogs block the ionic channel of the nicotinic receptor by two independent mechanisms. The first is a voltage- and time-dependent inactivation which occurs when the channel is in the resting (closed) state and the second a blockade of the open state of the ionic channel. Forward rate constants for open channel blockade ranged between 1 and 4 X $10^7$ $M^{-1}$ $sec^{-1}$. The reverse rate constant (for drug dissociation) was enhanced by analogs expected to have lower hydrophobicity. (Support: USPHS Grants NS-12063 and DA02804.)

247.13 **REMOVAL OF MAGNESIUM INDUCES NMDA RECEPTOR MEDIATED LTP BY TRIGGERING EPILEPTIFORM ACTIVITY.** Eric W. Harris and Carl W. Cotman, Dept. Psychobiology Univ. Calif., Irvine, CA 92717

NMDA (N-methyl-D-aspartate) preferring receptors are highly concentrated in stratum radiatum of hippocampal region CA1. NMDA receptor antagonists do not affect synaptic transmission along the Schaffer collateral input to this area, but do prevent the induction of synaptic long-term potentiation (LTP). $Mg^{++}$ blocks NMDA responses and synaptic responses mediated by NMDA receptors in the spinal cord. Recently, Coan and Collingridge (Neurosci. Lett, 53:21) reported that Schaffer responses were increased by removing $Mg^{++}$, and that a lasting potentiation persisted after reintroduction of $Mg^{++}$. The mechanisms underlying these effects have been investigated. We report that a late component of the Schaffer synaptic response is unblocked by removing $Mg^{++}$, and that the lasting potentiation is caused by epileptiform activity in the absence of $Mg^{++}$.

The selective NMDA receptor antagonists AP5 and AP7 have no effect on the Schaffer synaptic potential or the intracelluarly-recorded EPSP. Removal of $Mg^{++}$ increases the synaptic field potential and CA1 population spike, but the inital portion of the synaptic response is still AP5- and AP7-insensitive. A late component of the synaptic field potential in $Mg^{++}$-free medium is AP5- and AP7-sensitive, however. Addition of AP5 or AP7 also lessened repetitive spiking following Schaffer stimulation and reduced the amplitude of the CA1 population spike.

The lasting potentiation that persists after reintroduction of $Mg^{++}$ is indistinguishable from LTP produced by high frequency stimulation. If slices were first given high-frequency stimulation, there was no additional lasting potentiation after treatment with $Mg^{++}$-free medium. Similarly, after treatment with $Mg^{++}$-free medium, no further LTP was induced by high-frequency stimulation. Furthermore, like LTP, the lasting potentiation following treatment with $Mg^{++}$-free medium could be prevented, but not reversed, by AP5 or AP7. Lasting potentiation after $Mg^{++}$ removal was always preceeded by epileptiform activity in $Mg^{++}$-free medium. However, if CA1 was isolated from CA3 by transsection, even though removal of $Mg^{++}$ induced the AP5- and AP7-sensitive late synaptic response, there was reduced repetitive epileptiform activity and no lasting potentiation. Potentiation could be induced by high frequency stimulation in isolated CA1, or in isolated CA3 by treatment with $Mg^{++}$-free medium.

These results suggest that removing $Mg^{++}$ enhances the early portion of the Schaffer synaptic response by a mechanism unrelated to NMDA receptors (probably by increasing presynaptic release). Removal of $Mg^{++}$ also unblocks NMDA receptors mediating a late part of the synaptic response, which further enhances CA1 and CA3 cell discharge. Repetitive activation of Schaffer fibers results, thereby producing LTP. NMDA receptors are involved in the induction of LTP, but their activation after removal of $Mg^{++}$ does not directly produce LTP.

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247.14 EFFECT OF DIAZEPAM, PHENYTOIN, PENTOBARBITAL, AND PENTYLENETETRAZOL ON GABAERGIC INHIBITION MEASURED EXTRACELLULARLY BY PAIRED ORTHODROMIC STIMULATION IN RAT HIPPOCAMPUS IN VIVO. D. M. Rock* and C. P. Taylor. Dept. of Pharmacology, Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48105.

Extracellular field potentials (population spikes) were recorded from the hippocampus of urethane-anesthetized rats. A bipolar stimulating electrode was stereotaxically placed in the hippocampal commissure and an extracellular recording microelectrode was placed in the CA1 cell body region of the dorsal hippocampus. Identical pairs of monophasic pulses (0.5 msec duration, 10-1000 μA) were delivered to the hippocampal commissure. Stimulation of the commissure caused monosynaptic excitation of pyramidal cells and a resulting population spike (2-7 mV). When the delay between two stimuli of a pair was short (< 150 msec), GABAergic inhibition evoked by the first stimulus caused a decrement in the amplitude of the second population spike. Responses at various delays were taken before and after interperitoneal injections of drugs. In each experiment, eight data points were collected at an interstimulus delay resulting in roughly 50% inhibition of the second response. A two-tailed t-test was used to compare data before and after intraperitoneal drug administration. Doses of diazepam, pentobarbital and phenytoin were chosen in relation to previously-measured anticonvulsant activity in rat seizure models (tonic extensor seizures from maximal electroshock or subcutaneous pentylenetetrazol threshold seizures). Subconvulsant doses of pentylenetetrazol were also tested.

Diazepam (1, 4, and 8 mg/kg) and pentobarbital (7.5 and 15 mg/kg) significantly increased GABAergic inhibition in a dose-dependent manner as measured by decrement of the amplitude of paired orthodromic population spikes. Previous studies have shown that both diazepam and pentobarbital enhanced postsynaptic ionotphoretic GABA responses. In our study phenytoin (15 and 65 mg/kg) and pentylenetetrazol (20 mg/kg) both significantly decreased GABAergic inhibition. The current results with pentylenetetrazol confirm those of previous studies in which it attenuated postsynaptic GABA responses. However, some previous studies indicated that phenytoin enhanced GABAergic inhibition. The present results with phenytoin indicate that doses which prevented tonic extensor seizures from maximal electroshock in the rat did not enhance the amplitude or duration of hippocampal paired-pulse inhibition. We conclude that the anticonvulsant mechanism of phenytoin is fundamentally different from that of diazepam or of pentobarbital. However, the cellular mechanism responsible for the apparent decrease of inhibition by phenytoin is not known.

247.15 DITHIOTHREITOL INDUCES EPILEPTIFORM ACTIVITY IN THE GUINEA PIG HIPPOCAMPAL SLICE PREPARATION. J. M. Tolliver* and T. C. Pellmar (SPON: G. N. Catravas). Physiology Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814-5145.

The sulphydryl reducing agent dithiothreitol (DTT) can alter the uptake and release of GABA from slices of rat cerebral cortex (Iverson and Johnston, J. Neurochem. 18, 1939, 1971). Considering that this putative neurotransmitter may play a role in the normal function of the hippocampus, it was of interest to examine the effects of DTT on hippocampal function using extracellular recording techniques.

Antidromic and orthodromic population spikes were recorded from the CA1 cell body layer of guinea pig hippocampal slices in response to stimulation of the alveus and stratum radiatum, respectively. The synaptic response (pop PSP) to orthodromic stimulation was recorded from stratum radiatum. Recurrent inhibition was elicited by applying a conditioning stimulus to the alveus at selected times prior to orthodromic stimulation. Slices were superfused with DTT for 30 minutes and then washed with normal solution.

A 30-minute exposure to 0.5 mM DTT resulted in multiple spiking of the orthodromic response (epileptiform activity) (n = 20). This effect appeared within 25 to 40 minutes after starting DTT superfusion and continued without diminution for the length of the observation period (2 hours). The antidromic response was slightly increased in four slices and was not changed in two other slices. Increasing the DTT concentration to 1.0 mM reduced the onset delay of epileptiform activity (n = 3). At 2.0 mM, DTT induced epileptiform activity followed by a rapid loss of orthodromic and antidromic responses (n = 3). Input/output curves were generated to evaluate the site of DTT action. Epileptiform activity elicited by DTT (0.5 mM) was accompanied by an increased orthodromic spike amplitude for a given afferent volley amplitude or pop PSP slope (n = 7). In four of the seven slices, the pop PSP slope evoked by a particular afferent volley size was increased by DTT. The effect of DTT on recurrent inhibition was also examined. DTT (0.5 mM) significantly reduced recurrent inhibition elicited at interpulse intervals of 1 and 3 msec by 25% and 29%, respectively (n = 7). These observations suggest that DDT induces epileptiform activity predominately via postsynaptic mechanisms and possibly also by synaptic mechanisms. Possible effects of DTT currently being considered include reduced recurrent inhibition, membrane depolarization and elevated postsynaptic sensitivity to putative, excitatory neurotransmitters in CA1 pyramidal cells.

247.16 ADENOSINE INHIBITS HIPPOCAMPAL EPILEPTIFORM ACTIVITY. B. Ault* and C.M. Wang* (SPON: A. Tadepalli). Dept. of Pharmacology, Wellcome Research Laboratories, Burroughs Wellcome Co., Research Triangle Park, NC 27709.

Burst firing induced in hippocampal area CA3 by bicuculline, kainic acid or tetanic electrical stimulation provides a convenient model of limbic seizure activity. We have found that the $GABA_B$ receptor agonist baclofen selectively depresses such activity. Since recent data have suggested that adenosine and baclofen-sensitive receptors are linked to a common second messenger system, we have examined the effect of purinergic agonists upon hippocampal epileptiform activity and studied the role of endogenous adenosine.

Extracellular field potentials were recorded from the CA3 pyramidal cell body layer of transverse hippocampal slices (425 μ nominal thickness). Burst activity was induced at rates between 4 and 22/min (means ± S.E.M. of 10.4 ± 0.6 bursts/min, N=49) by addition of bicuculline methiodide (50 μM) to the medium and slightly elevating the $K^+$ concentration (5 mM).

Purinergic agonists could completely suppress burst firing and the following $IC_{50}$ values were determined from dose-response curves: adenosine, 1.5 ± 0.2 μM N=10; 2-chloroadenosine, 0.2 ± 0.07 μM N=3; L-phenylisopropyladenosine, 13.4 ± 2.3 nM N=4; cyclohexyladenosine, 7.9 ± 1.5 nM N=4. These concentrations are approximately tenfold lower than those necessary to inhibit electrically-evoked synaptic potentials in area CA1, which parallels the relative potency difference noted for baclofen.

Theophylline (30 μM) increased the rate of burst firing in the presence of bicuculline and antagonized the effect of exogenous adenosine with a mean dose ratio of 7.5 ± 1.6, N=3. Similar studies using baclofen as an agonist indicated that about half of the antagonism was due to the increase in firing rate. The adenosine uptake inhibitor dipyridamole (0.03 - 1.0 μg/ml) decreased the rate of burst firing. Hippocampal slices in normal medium did not normally show bursting activity. In these preparations, superfusion of theophylline (30 μM) induced burst activity (N=5).

These data show that purinergic agonists are potent inhibitors of epileptiform activity in hippocampal area CA3 and that endogenous adenosine tonically serves to prevent the generation of burst discharges. A deficit in purinergic inhibition could therefore be important in the tiology of limbic seizures.

248.1 EFFECTS OF PHORBOL ESTER ON QUANTAL ACETYLCHOLINE RELEASE AT FROG NEUROMUSCULAR JUNCTION. C. Haimann * (SPON: European Neuroscience Association). CNR Ctr. of Cytopharmacol., Dept. of Pharmacol. and Ctr. Peripheral Neuropathies and Neuromuscular Diseases, 20129 Milano, Italy.

The possible involvement of protein kinase C in quantal transmitter release was studied at frog neuromuscular junction by using a phorbol ester that mimics the physiological activation of this protein by endogenous diacylglycerol (for ref. Nishizuka, Y., Nature, 308: 693, 1984).

Standard electrophysiological techniques were used to record intracellularly miniature endplate potentials (mepps) and endplate potentials (epps) at frog neuromuscular junctions soaked in modified Ringer's solution (0.7 mM $Ca^{2+}$ and 4 mM $Mg^{2+}$) containing 12-O-tetradecanoyl-phorbol-13 acetate (TPA), dissolved with anhydrous dimethyl-sulfoxide (DMSO). The quantal content of the epp was estimated from the ratio of the mean amplitudes of epps and mepps, obtained by averaging several mepps and epps evoked by nerve stimulation at low frequency (0.5 Hz). TPA was applied directly into the experimental chamber at final concentrations ranging from $1 \times 10^{-7}$ M to $3 \times 10^{-6}$ M. TPA induced an increase of the quantal content of about 3 fold. The minimal concentration necessary to elicit an effect was about $6 \times 10^{-7}$ M and the magnitude of the effect was apparently not correlated to the increase in concentration up to $3 \times 10^{-6}$ M. The enhancement of the evoked release developed slowly, reached its maximum in about 60 min and was partially reversible by washing the preparation with the modified Ringer's solution. After this washing period a second addition of TPA did not induce any further increase in quantal content. By contrast, TPA did not significantly affect mepp frequency or amplitude. The lack of effect of mepp amplitude suggests that the observed increase in epp amplitude is not due to gross changes in postsynaptic sensitivity. DMSO was uneffective in modifying quantal content in the range used (0.1-0.3%). Some neuromuscular preparations were also exposed to the TPA analogue 4-alpha-phorbol-12,13 didecanoate, which is unable to activate protein kinase C. This compound did not induce any change in the quantal content of epp or in mepp parameters at concentrations up to $2 \times 10^{-5}$ M.

These findings suggest that the increase in quantal release of acetylcholine evoked by nerve stimulation is due to the activation of protein kinase C. If this suggestion is correct it would imply a modulatory role of protein kinase even at those synapses such as neuromuscular junctions with high safety factor for transmitter release.

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248.2 CHARACTERIZATION OF PHORBOL ESTER-STIMULATED PHOSPHORYLATION OF SPECIFIC PROTEINS IN RAT BRAIN SYNAPTOSOMES. J.K.T. Wang*, S.I. Walaas and P. Greengard. (SPON:R. Lewis). Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, New York, NY 10021.

The $Ca^{++}$/phospholipid-dependent protein kinase (kinase C) is present in synaptic regions of brain and can be specifically activated by phorbol esters. We now report the characterization of the phosphorylation of proteins in phorbol ester-treated synaptosomes, and a comparison with the phosphorylation induced by $K^+$-depolarization.

Rat cerebral cortical synaptosomes were prelabelled with $^{32}P_i$ and exposed to phorbol esters or to elevated $K^+$. Upon electrophoresis on two-dimensional SDS/polyacrylamide gel, a number of phosphoproteins from phorbol ester-treated synaptosomes showed increased incorporation of radiolabel. The two major substrates were acidic proteins of 87-kilodalton (kDa) and 50kDa. The 87kDa protein, previously reported to be a specific substrate for kinase C and to be enriched in brain (Wu, W.C.-S. et al., PNAS, 79:5249, 1982), was used as a specific marker for kinase C activation. Phorbol esters stimulated the phosphorylation of this protein in a time- and concentration-dependent manner, with the potency series (phorbol 12,13-dibutyrate = phorbol 12-myristate 13-acetate > phorbol 12,13-diacetate >> phorbol 13-acetate > phorbol) being similar to that observed in other systems. Extracellular $Ca^{++}$ was not required for this effect. Most of the substrates stimulated by phorbol esters were also sensitive to $K^+$-depolarization, although the degree of stimulation was less in the latter case. The major difference between the two was that elevated $K^+$, by activating the $Ca^{++}$/calmodulin-dependent (CaM) kinases, phosphorylated nerve terminal vesicle-associated proteins synapsin I and protein III. Phorbol esters, in contrast, had no effect on these proteins. Recent studies have shown that activation of the CaM-kinase system may play a critical regulatory role in neurotransmission at the squid giant synapse (Llinas, R. et al., PNAS, in press). However, kinase C is also activated during depolarization (Wu et al., 1982; present results). The following abstract by Nichols et al. shows that treatment of brain synaptosomes by a phorbol ester results in the modulation of $Ca^{++}$-dependent, $K^+$-induced neurotransmitter release. It is therefore possible that kinase C, by acting on one or more of the substrates described in this report, provides an additional mechanism of regulation of neurotransmission.

248.3 PHORBOL ESTER ENHANCES CALCIUM-DEPENDENT NEUROTRANSMITTER RELEASE FROM RAT BRAIN SYNAPTOSOMES. R.A. Nichols, J.W. Haycock, J.K-.T. Wang* and P. Greengard. Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, New York, NY 10021.

Depolarization of the synaptic terminal modulates the state of phosphorylation of specific phosphoproteins in a $Ca^{++}$-dependent manner. These changes are largely mediated by $Ca^{++}$-dependent protein kinases, a number of which have been identified. The preceding abstract (Wang, et al) describes the phosphorylation of proteins in synaptosomes treated with phorbol esters, compounds known to activate the $Ca^{++}$/phospholipid-dependent protein kinase (kinase C). Those studies demonstrated that most but not all of the synaptosomal phosphoprotein substrates affected by $K^+$-stimulated depolarization are also modulated by phorbol esters. The results confirmed the finding that kinase C is activated during depolarization of the presynaptic terminal (Wu, et al., PNAS, 79: 5249, 1982). The present study addressed the potential role of kinase C in the regulation of neurotransmitter release from synaptosomes by examining the effects of phorbol esters.

Pretreatment of rat brain synaptosomes in a continuous perfusion system with phorbol-12,13-dibutyrate (PDBu) led to an increase in $K^+$-stimulated, $Ca^{++}$-dependent release of neurotransmitter from prelabeled neurotransmitter pools. An increase was observed at all $Ca^{++}$ concentrations studied (0.05 to 3 mM). At 0.5 mM extracellular $Ca^{++}$, PDBu caused an average increase of 30% (range: 16-66%) in neurotransmitter release. The effect was dependent upon the concentration of PDBu. The PDBu-stimulated increase was observed for all brain regions and neurotransmitters studied (cerebral cortex: norepinephrine, acetylcholine and gamma-aminobutyric acid; corpus striatum: dopamine and acetylcholine; hippocampus: norepinephrine). PDBu had a slight effect (approx. 8%) on $K^+$-stimulated neurotransmitter efflux measured in the absence of extracellular $Ca^{++}$. Net accumulation of neurotransmitter was unaffected by the presence of PDBu. Inactive phorbol esters, such as phorbol, did not affect $K^+$-stimulated, $Ca^{++}$-dependent release. The PDBu-stimulated increase in release was also observed when veratridine or A23187 were used as secretagogues.

As the degree of enhancement of neurotransmitter release by phorbol ester was dependent upon extracellular $Ca^{++}$ and was observed with veratridine and A23187 as well as with $K^+$-stimulated depolarization, we postulate that activation of kinase C in the synaptic terminal results in the modulation of the stimulus-secretion coupling mechanism.

248.4 PHORBOL DIBUTYRATE INCREASES PROTEIN III PHOSPHORYLATION IN BOVINE ADRENAL CHROMAFFIN CELLS. J.W. Haycock, M.D. Browning and P. Greengard. Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, New York, NY 10021.

Proteins IIIa and IIIb (referred to collectively as Protein III) are members of a family of synaptic vesicle-associated phosphoproteins present in central and peripheral neurons. Protein III is also present in isolated bovine adrenal chromaffin cells. As previously described (Haycock, J.W., et al., Soc. Neurosci. Abstr., 10: 196, 1984), Protein III phosphorylation is increased by acetylcholine (ACh) and other secretagogues. The protein kinase(s) involved in this effect have not, however, been determined. The present studies investigated the possible involvement of two protein kinase systems: cAMP-dependent protein kinase (kinase A) and calcium/phospholipid-dependent protein kinase (kinase C) in the phosphorylation of Protein III in chromaffin cells.

After incubation of the chromaffin cells with $^{32}PO_4$, treatment of the chromaffin cells with phorbol dibutyrate (10-1000 nM) led to an increase in Protein III phosphorylation. Because the phosphorylation of Protein III by treatment with phorbol ester and ACh was not additive, it seemed possible that ACh and the phorbol ester might increase Protein III phosphorylation via common processes.

In that phorbol esters are thought to specifically activate kinase C, a possible role for kinase C in the phosphorylation of Protein III was investigated. Protein III was extracted (Zn acetate precipitation, pH 3 extraction) from treated and untreated chromaffin cells, and the ability of purified, exogenous kinase A (for which Protein III is known to be a substrate) or purified, exogenous kinase C to phosphorylate Protein III was determined. Treatment of the cells with either ACh or phorbol dibutyrate led to a decrease in the phosphorylation of Protein III by kinase A, indicating that both treatments led to an increase in situ in occupation of phosphate acceptor sites accessible to kinase A in vitro. However, kinase C was at least two orders of magnitude less effective than kinase A in phosphorylating Protein III. The fact that Protein III was not an effective substrate for kinase C in vitro suggests that phorbol dibutyrate may enhance Protein III phosphorylation in chromaffin cells indirectly via activation of cAMP-dependent protein kinase.

248.5 EVIDENCE FOR 4 CALCIUM ACTIVATED SITES INVOLVED IN TRANSMITTER RELEASE AND FACILITATION AT THE SQUID GIANT SYANPSE. E.F. Stanley, NINCDS Biophysics Laboratory, MBL, Woods Hole, MA.

Transmitter release has been reported to be proportional to a high power (N) of external Ca, suggesting that several Ca ions cooperate in the release of each transmitter quantum (Dodge and Rahamimoff, 1967). The squid giant synapse has recently been shown to have a high N value of 4 at low stimulus frequencies (Stanley, Biol Bull 167:532, 1984). Repetetive electrical stimulation of the prenerve results in facilitation of the EPSP recorded in the postsynaptic giant axon at stimulus frequencies above 0.3Hz. This facilitation has been attributed to a decline in the number of Ca ions required to activate the transmitter release mechanism: at 1Hz, N=3; 20Hz, N=2; and 80Hz, N=1 (Stanley, Biophys J. 47:55a). The dependence of facilitation on the stimulus frequency was examined in detail in this study in order to examine the transitions from N=4 to N=1.

The stellate ganlion was mounted in a chamber and continually perfused through its aorta, a technique that allows the rapid and controlled exchange of external solutions. The perfusate was artificial sea water containing low Ca (2mM) so that singe EPSPs were reduced to less than 2mV. At this low level of release the transmitter release mechanism is not saturated and the EPSP amplitude follows the fourth power of external Ca. In addition, transmitter depletion during repetetive stimulation is negligable at all but the highest stimulus frequencies. At each frequency tested, a train of stimuli was given to the prenerve until facilitation of the EPSP reached a maximum. The maximum facilitated EPSP was expressed as a percentage of the innitial EPSP in the train. Stimulus frequencies ranged from 0.1 to 90Hz.

The relation between maximum facilitation and stimulus frequency is best described by a curve with three discreet steps the plateaus of which occur at 1-3, 20-40, and >80Hz. It is suggested that each step corresponds to the activation of one of the four Ca-activated sites involved in the quantal release mechanism: thus, at any stimulus frequency the number of Ca sites activated (steps) plus the number of additional Ca ions required to trigger transmitter release (N) equals 4. These results suggest that the activation of transmitter release by Ca involves the triggering of a sequence of identifiable events, requiring the cooperative net action of four Ca ions for completion. Facilitation can be attributed to the activation of the release mechanism to a subthreshold level by the binding of one to three ions.

248.6 PATHOPHYSIOLOGY OF THE LAMBERT-EATON MYASTHENIC SYNDROME: EVIDENCE FOR CALCIUM CHANNEL BLOCKADE. Yong I. Kim, Depts. of Neurology and Biomedical Engineering, Univ. of Virginia School of Medicine, Charlottesville, VA 22908.

Previous studies from our laboratory have indicated that the electrophysiologic features of the Lambert-Eaton myasthenic syndrome (LES) are faithfully reproduced when the human disorder is passively transferred to mice (Kim, Muscle Nerve 8:162-172, 1985, Kim et al., Soc. Neurosci. Abstr. 10:199, 1984). In the present study the subsynaptic mechanism underlying LES was examined using the mouse passive transfer model.

Mice were injected daily (>20 days) with whole plasma or immunoglobulin fractions obtained from the controls and LES patients. In LES animals, quantal content ($m_d$), measured with 1.2 mM $Ca^{2+}$ and 10 mM $Mg^{2+}$, was markedly reduced. The increase in MEPP frequency ($F/F_o$ = test/control) occurring in response to high $K^+$ (17.5 mM) was also partially prevented. Since the elevation of $[K^+]_o$ activates voltage-dependent $Ca^{2+}$ channels and promotes $Ca^{2+}$ influx by depolarizing the motor nerve terminal, this observation alone suggests that $Ca^{2+}$ channels may be blocked at LES terminals, mimicking the action of $Ca^{2+}$ antagonists such as $Mg^{2+}$ and $Co^{2+}$. In this regard, treatment of

| Mean±SEM [No. of fibers] *p<0.001 | Quantal Content ($m_d$) | Relative MEPP Frequency ($F/F_o$) | | |
|---|---|---|---|---|
| | | $K^+$ | LiCl | Sucrose |
| Control | 2.01±0.11[112] | 107±4[115] | 147±8[97] | 131±14[25] |
| LES | 0.42±0.02[107]* | 34±2[99]* | 150±7[95] | 125±10[27] |

normal junctions with 0.25-0.3 mM $CoCl_2$ also reproduced similar presynaptic impairments. This "calcium channel" hypothesis, however, is not compelling because other alternative mechanisms such as abnormal intraterminal $Ca^{2+}$ buffering capacity or reduced ACh release sites can also explain the defects in LES. To clarify these points, the effects on $F/F_o$ of A) 135 mM LiCl (replacing NaCl) and B) 400 mM sucrose (hyperosmosis) were examined under $Ca^{2+}$-free conditions in the presence of 1 mM EGTA. These procedures are thought to stimulate the quantal discharge by increasing intraterminal $[Ca^{2+}]_i$ independently of external $Ca^{2+}$. The resulting MEPP discharges in both cases were similar at the control and LES junctions. Experiments on $CoCl_2$-treated junctions yielded similar data.

These results provide electrophysiologic evidence that the presynaptic impairment in LES is caused by reduced $Ca^{2+}$ entry secondary to the antibody-induced blockade of the voltage-dependent $Ca^{2+}$ channels. (Supported by NIH grant NS18607 and a research grant from the Muscular Dystrophy Association).

248.7 SUPPRESSION OF SYNAPTOSOMAL $^{45}Ca$ UPTAKE BY METHYLMERCURY. William D. Atchison, Urmila Joshi* and John E. Thornburg. Dept. Pharmacol./Toxicol., Michigan State Univ., East Lansing, MI 48824.

Acute administration of methylmercury (MeHg) suppresses synaptic transmission by a presynaptic block of transmitter release. One potential mechanism by which this block may occur is through suppression of voltage-dependent $Ca^{2+}$ entry into the nerve terminal in response to the action potential. Nachshen and Blaustein (J. Gen. Physiol. 83, 941-967, 1984) reported that inorganic, divalent mercury ($HgCl_2$) suppressed the uptake of $^{45}Ca$ during $K^+$-induced depolarization or following predepolarization in rat brain synaptosomes. The objective of the present study was to determine whether MeHg, which is a monovalent cation would also suppress $^{45}Ca$ uptake into synaptosomes. If so, this might explain in part the block of nerve evoked transmitter release reported previously with MeHg (Atchison and Narahashi, Neurotox. 3(3), 37-50, 1982). Synaptosomes were prepared from rat forebrains using conventional methods. The effects of (10-500 µM) MeHg on total $^{45}Ca$ uptake in $K^+$ depolarized ($[K^+]$ = 41 mM) synaptosomes was measured, and compared to that obtained in the presence of $HgCl_2$ (10-500 µM). Inorganic mercury produced a concentration-dependent suppression of depolarization-induced $^{45}Ca$ uptake. Peak inhibition occurred at 200 µM $HgCl_2$, which suppressed $^{45}Ca$ uptake to approximately 5% of premercury control values. These results corroborate those obtained previously by Nachshen and Blaustein. Similarly, MeHg also decreased total $^{45}Ca$ uptake, although the maximal inhibition produced (70% at 200 µM) was less than that produced by $HgCl_2$. The effect of MeHg was more apparent following 10 sec incubation in synaptosomes predepolarized for 10 sec with 41 mM $K^+$ prior to MeHg and $^{45}Ca$ addition than following a 1 sec incubation. A significant decrease in $^{45}Ca$ uptake occurred at 200 and 500 µM MeHg following the 10 sec incubation. $^{45}Ca$ uptake following a 1 sec incubation was also depressed by MeHg, but the decrease was not statistically significant. As Ca uptake during 10 sec is thought to represent flux through slow, non-inactivating Ca channels it appears that the effect of MeHg is most pronounced at this site. The total uptake of $^{45}Ca$ as a function of extracellular Ca in the absence of MeHg saturated at approximately 0.5 mM = $[Ca]_o$. When 200 µM MeHg was present, $^{45}Ca$ uptake also saturated at 0.5 mM $Ca^{2+}$ although the total uptake was only 35-40% of that in the absence of MeHg. Thus, MeHg appears to behave as a noncompetitive, irreversible inhibitor to Ca entry through a slow noninactivating membrane channel. These results are consistent with the block of Ca-dependent, nerve-evoked transmitter release induced by MeHg at the vertebrate neuromuscular junction. (Supported by NIH grant ES03299.)

248.8 FLUOXETINE INHIBITS $[^3H]$-5-HT RELEASE IN RAT SPINAL CORD SYNAPTOSOMES: POSSIBLE $Ca^{2+}$ CHANNEL ANTAGONISM. K.A. Stauderman* and D.J. Jones, Departments of Pharmacology and Anesthesiology, The University of Texas Health Science Center, San Antonio, TX 78284

Fluoxetine has been shown to be a selective and potent inhibitor of neuronal 5-HT uptake systems. However, this compound has a demonstrated ability to inhibit $K^+$-induced $[^3H]$-5-HT release in synaptosomes. Whether this inhibitory effect is mediated through a site-specific interaction is not known. Consequently, experiments were conducted to illuminate the mechanism of fluoxetine-induced inhibition of $K^+$-stimulated $[^3H]$-5-HT release.

$P_2$ fractions obtained from the spinal cords of male Sprague-Dawley rats were incubated at 37°C for 15 min in a buffer solution containing 100 uM iproniazid and 50 nM $[^3H]$-5-HT. Tissue aliquots were then loaded onto filters placed at the bottom of superfusion chambers, washed, and superfused for 30 min with a resting superfusion buffer (containing 3 mM $K^+$). Synaptosomes were then depolarized with either a 15 or 30 mM $K^+$ buffer for 15 min. After depolarization, $[^3H]$-5-HT remaining in the synaptosomes was extracted. Fractions were collected every 2 min. $[^3H]$-5-HT release was expressed as a percentage of total $[^3H]$-5-HT present before collection of each fraction. Test drugs and conditions were present from the beginning of superfusion.

$K^+$-induced $[^3H]$-5-HT release from rat spinal cord synaptosomes, determined as the difference from resting release values, was both $Ca^{2+}$-dependent and dependent on the concentration of $K^+$. At normal $[Ca^{2+}]$ (1.25 mM), 1 uM fluoxetine produced a 35% inhibition of 15 mM $K^+$-induced release and a 17% inhibition of 30 mM $K^+$-induced release. When the $[Ca^{2+}]$ was reduced to 0.125 mM, 1 uM fluoxetine decreased 30 mM $K^+$-induced release 46%. The $Ca^{2+}$-dependence of fluoxetine inhibition suggested the effect may involve presynaptic autoreceptors which regulate 5-HT release and are coupled to voltage-dependent $Ca^{2+}$ channels. Therefore, the effects of agents which alter autoreceptor function were tested in the absence and presence of 1 uM fluoxetine. 5-HT (30 nM) reduced 15 mM $K^+$-induced $[^3H]$-5-HT release by 30-40% and this effect was blocked by methiothepin (100 nM), a 5-HT receptor antagonist. However, methiothepin did not alter the inhibition of release produced by 1 uM fluoxetine. Thus, it appears that fluoxetine is not a 5-HT autoreceptor agonist. Considering fluoxetine's low affinity for neurotransmitter receptors and the structural similarity between fluoxetine and dihydropyridine $Ca^{2+}$ channel antagonists, it is proposed that fluoxetine blocks $Ca^{2+}$ channels, thus inhibiting $[^3H]$-5-HT release in a $Ca^{2+}$-dependent fashion.

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248.9 SUBSTANCE B: AN ANTAGONIST OF PURINERGIC, MUSCARINIC AND ADRENERGIC PRESYNAPTIC RECEPTOR-MEDIATED INHIBITION OF [$^3$H]ACETYLCHOLINE RELEASE FROM SYNAPTOSOMES. L.B. Pearce*, C.G. Benishin and J.R. Cooper. Dept. Pharmacology, Yale Univ. Sch. Med., New Haven, CT 06510.

Presynaptic receptor-mediated control of neurotransmitter release although well documented in peripheral systems, has been more difficult to demonstrate in brain, particularly brain synaptosome preparations. While purinergic receptor-mediated inhibition of transmitter release has been demonstrated using electrophysiological recording and iontophoresis or measurement of transmitter release from brain slices, effects on release of [$^3$H]acetylcholine (ACh) from synaptosomes have not been well established. We have observed that guinea pig and rat whole brain extracts contain a factor which we have termed substance B that reverses presynaptic receptor-mediated inhibition of [$^3$H]ACh release. Crude synaptosomes were isolated from guinea pig ileum myenteric plexus and preloaded with [$^3$H]choline. The effect of various presynaptic receptor agonists on dimethylphenylpiperazinium (DMPP)-evoked release of [$^3$H]ACh was quantitated. When extracts of guinea pig brain were added to these assays, inhibition of ACh release produced by (-) phenylisopropyl adenosine, oxotremorine and clonidine was reversed. The factor did not effect evoked release of the transmitter. This factor was found to be associated with purified synaptosomes prepared from both guinea pig and rat brain. Substance B is insensitive to treatment with pronase, phospholipase C or boiling for 30 min., whereas ashing destroyed activity. Gel chromatography of an ultrafiltrate (10,000 MW cutoff) of a brain extract suggests that substance B has a molecular weight of approximately 700. While the nature of substance B remains unclear it is evident that this brain factor reverses presynaptic purinergic, muscarinic, and $\alpha$-adrenergic receptor-mediated inhibition of [$^3$H]ACh release from crude synaptosomes which may explain the difficulty encountered in our laboratory as well as that of others in attempting to demonstrate presynaptic mechanisms in brain. The significance of substance B in control of presynaptic regulatory mechanisms remains to be established. Results, however, of experiments using an intact tissue preparation confirm and extend the above findings (see abstract by Benishin et al.) suggesting a potentially important function.

248.10 SUBSTANCE B INTERFERES WITH MODULATION OF NEURONAL ACTIVITY IN THE INTACT LONGITUDINAL MUSCLE-MYENTERIC PLEXUS OF GUINEA PIG. C.G. Benishin, L.B. Pearce*, and J.R. Cooper. Dept. of Pharmacology, Yale Univ. Sch. Med., New Haven, CT 06510.

The guinea pig ileum myenteric plexus has proven to be a useful model for elucidating cholinergic mechanisms. For example, a variety of agents are capable of modulating the release of acetylcholine (ACh) in the myenteric plexus, whereas these modulatory mechanisms are much more difficult to demonstrate in the brain. Evidence presented in the previous abstract (Pearce et al.) indicates the presence of a substance in brain (Substance B) which prevents the modulation of evoked [$^3$H]ACh release from synaptosomes prepared from guinea pig longitudinal muscle-myenteric plexus. In the present study intact strips of longitudinal muscle-myenteric plexus were suspended in a tissue bath and field stimulated to elicit maximal contractions. Several drugs were used to inhibit the force of contraction including $\alpha_2$ adrenergic and purinergic agonists (clonidine and 2-chloroadenosine, respectively) which are postulated to act via presynaptic mechanisms, as well as agents whose actions are less clearly defined (e.g. morphine and PCP). An extract of brain which was previously shown to reverse the inhibitory effects of $\alpha_2$ adrenergic and purinergic agonists on [$^3$H]ACh release from ileal synaptosomes, was capable of a dose-dependent reversal of the inhibition of contractions by purinergic and $\alpha_2$ adrenergic receptor agonists in the intact longitudinal muscle-myenteric plexus preparation. The brain extract also reversed opiate receptor mediated inhibition of contractions in a dose-dependent manner. Substance B had a minimal effect on electrically induced contractions in the absence of modulation.

Several results suggest that myenteric plexus neurons may contain a small quantity of Substance B which is washed out early in the experiments. Therefore the intact guinea pig longitudinal muscle-myenteric plexus appears to be a suitable bioassay system for quantifying and examining the properties of this agent.

248.11 SELECTIVE ALTERATION OF DOPAMINE AND ACETYLCHOLINE RELEASE DURING ANOXIA OR 3,4-DIAMINOPYRIDINE TREATMENT. G.B. Freeman, V. Mykytyn* and G.E. Gibson. Cornell Univ. Med. Coll., Burke Rehab. Ctr., White Plains, NY 10605.

Hypoxia (i.e. low oxygen) reduces neurotransmitter metabolism through an unknown mechanism. In cortical slices, hypoxia reduces acetylcholine (ACh) release, stimulates glutamate release, but does not alter norepinephrine, serotonin, or $\gamma$-aminobutyrate release. The hypoxic-induced decline in ACh synthesis appears to be linked to altered calcium homeostasis. To further investigate the relative susceptibility of neurotransmitters to impaired oxidative metabolism, the in vitro release of glutamate, dopamine and ACh were determined in mouse brain striatal slices during anoxia. After a 60-min preincubation, the potassium-stimulated (31 mM-KCl) release of dopamine was determined at 0, 0.5, 1.0, 2.5, 5.0 and 10 min. Dopamine release increased 12-fold after 5 minutes and then decreased. Thus, subsequent experiments utilized 5 min time intervals. The potassium-stimulated release of dopamine and ACh in this system were calcium-dependent whereas glutamate release was not. In 31 mM-KCl, 2.3 mM calcium media, anoxia (100% $N_2$) increased dopamine and glutamate release, 56% and 55%, respectively, but decreased acetylcholine release 22%. The omission of calcium during an anoxic incubation with high $K^+$ failed to abolish the increased release of dopamine, while it further decreased ACh release and further increased glutamate release. The selective alteration in the release of these neurotransmitters with anoxia and the previously demonstrated impairment of calcium uptake by anoxia suggest that calcium's role in their release varies. Thus, the effects of 3,4-diaminopyridine, a drug which promotes calcium influx into nerve endings, were investigated. In low potassium media (5 mM-KCl), 100 uM of 3,4-diaminopyridine increased ACh release (+20%) to levels similar to potassium-stimulated samples. In contrast, dopamine release was markedly reduced (-42%). In summary, anoxia impairs ACh release and stimulates dopamine release, whereas 3,4-diaminopyridine has the opposite effect. A variable role of calcium in neurotransmitter metabolism in response to injury or drug treatment may underlie these differences. (Supported by grants NS03346, AG04171, AG05352 and the Burke Relief Foundation.)

248.12 EFFECTS OF DIVALENT CATIONS ON EXCITATORY AND INHIBITORY POTENTIALS IN THE RAT HIPPOCAMPUS IN SITU. J. Casullo* (SPON: K. Krnjević) Dept. Anaesthesia Research, McGill University, Montréal, Québec H3G 1Y6, Canada.

Experiments were performed on rats anaesthetized with urethane or dial and maintained in a stereotaxic head frame. Extracellular field potentials were recorded in the CA1 region of the dorsal hippocampus with the central barrel of 5-barrelled micropipettes. Peripheral barrels were used for iontophoretic applications of 0.1 M $CaCl_2$, $MgCl_2$, $MnCl_2$, or $CoCl_2$. In some experiments somatic and apical dendritic potentials were recorded simultaneously with two multibarrelled pipettes separated by a vertical inter-tip distance of $\approx$ 250 µm.

Fimbrial stimulation (0.8 Hz) at intensities below threshold for evoking a population spike in CA1, induces excitatory and inhibitory postsynaptic currents in pyramidal cells, which generate negative and positive extracellular potentials in stratum radiatum (SR) and stratum pyramidale (SP) respectively. During simultaneous recording from SR and SP, divalent cations applied in either SR or SP produced changes only in the field response recorded at the same level indicating that negative and positive field potentials arise essentially independently. Both types of PSP field responses were consistently potentiated by $Ca^{2+}$ and depressed by $Mg^{2+}$, $Mn^{2+}$, and $Co^{2+}$. Typically, for current applications of 50-100 nA lasting 30 s, $Ca^{2+}$ increased the amplitude of the negative and positive waves by 40% and 20% respectively, while $Mg^{2+}$, $Mn^{2+}$, and $Co^{2+}$ reduced the amplitude of the negative and positive waves by 30-50% and 50-80% respectively. In addition to differences in magnitude there may also be differences in the time course of effects on excitatory and inhibitory potentials when cations are applied at different levels in CA1.

These results suggest that transmitter release from both excitatory and inhibitory terminals can be enhanced by locally applied $Ca^{2+}$ and reduced by locally applied $Mg^{2+}$, $Mn^{2+}$, or $Co^{2+}$. Indeed, when a population spike was evoked by stronger fimbrial stimulation, its amplitude could be depressed by $Ca^{2+}$ and increased by $Mg^{2+}$, $Mn^{2+}$, and $Co^{2+}$ when the cations were applied in SP. Conversely, the spike amplitude was augmented by $Ca^{2+}$ and reduced by the other ions when the cations were released in SR.

Supported by the Medical Research Council of Canada.

248.13 CENTRAL SYNAPTIC EFFECT AND CONVULSIONS PRODUCED BY RUTHENIUM RED INTRAPERITONEALLY INJECTED TO ADULT RATS. A. Feria-Velasco, J. Arauz-Contreras*, A. Palomera* and A. Morales*. Div. of Neurobiology, Unidad de Investigación Biomédica de Occidente, I.M.S.S.. Guadalajara, Jal. MEXICO.

Ruthenium red (RR), a polycationic, hydrosoluble dye combines with several substances at the external surface of cells, including those participating in the configuration of the voltage-dependent $Ca^{++}$ channel. RR when injected intraperitoneally (i.p.) to mice produces flaccid paralysis, whereas when administered intracisternally to mice and cats induces convulsions (Tapia, R. et al.: Brain Res.,116:101, 1976; Kubli-Garfias, C. et al: Neurosci., 7:2777, 1982). In our laboratory when RR has been injected i.p. to adult rats induced hyperexcitability and convulsions after a flaccid paralysis period, suggesting a central effect of the dye (Arauz-Contreras, J. et al., Arch. Inv. Med.,13:271, 1982).

In the present work a systematic study of motor activity, correlated with electrocorticographic recordings (ECG) was carried out following i.p. administration of RR (5-30 $mg.Kg^{-1}$) to adult rats. Synaptosomal ($P_2$) fraction from brains of rats injected with 12.5 and 20 $mg.Kg^{-1}$ RR was obtained at various time intervals after RR administration, to demonstrate ruthenium (Ru)signal by means of X-ray spectrometric analysis (EDAX) on a system adapted to a scanning electron microscope. In corresponding experiments, high affinity $Na^+$-dependent uptake of radiolabeled catecholamines, and $Ca^{++}$-dependent [$^{14}C$] dopamine release induced by depolarizing stimuli, from $P_2$ fraction obtained from rats i.p. injected with 12.5 $mg.Kg^{-1}$ RR were measured.

Rats i.p. injected with RR, showed the previously communicated changes in motor activity in a dose-dependent manner (Arch. Inv. Med.,13:271, 1982). No variations were seen in the ECG during the period of flaccid paralysis, whereas the electromyographic recording (EMG) showed a marked voltage reduction. Typical trains of high voltage spikes were seen in the ECG during the convulsive period, which was correlated with hyperactivity in the EMG. Ru signal in $L\alpha$ and $L\beta$ shells was detected in the EDAX of $P_2$ fraction obtained from rats injected i.p. with RR at 2 and 4 hours after RR administration, and no significant changes were seen in the high affinity $Na^+$-dependent uptake of catecholamines by that $P_2$ fraction. However, the $Ca^{++}$-dependent release of [$^{14}C$] dopamine induced by depolarizing stimuli (high potassium or veratrine) was significantly reduced in the brain $P_2$ fraction obtained from rats injected i.p. with RR.

It can be concluded that besides the peripheral effect of RR in rats, the convulsive episode observed afterwards in a dose-dependent manner is produced by a central action of RR at synaptic level, probably diffusing from areas devoided of blood-brain-barrier. This subject is under investigation in our laboratory.

## POSTSYNAPTIC MECHANISMS II

249.1 GABA-ACTIVATED CHANNELS IN CULTURED CHICK CEREBRAL NEURONS. D. S. Weiss*, E. M. Barnes, Jr.*, and J. J. Hablitz. Depts.of Neurology and Biochemistry and Program in Neuroscience, Baylor Col. of Med., Houston, TX 77030.

To understand the mechanism of the postsynaptic action of the inhibitory neurotransmitter GABA, quantitative physiological measurements of the interaction of the agonist with its receptor are needed. We have approached this problem using cultured neurons from the chick cerebrum. These cultures are essentially glia-free for the first 8-10 days, and the neurons are predominantly GABAergic, as shown by the presence of GAD-like immunoreactivity. Previous studies (Thampy & Barnes, 1984) have shown the existence of a GABA-gated chloride flux in these neurons which is antagonized by picrotoxin and bicuculline. The present electrophysiological studies have identified and begun to characterize functional GABA synapses and channels in these cultures.

The gigaohm patch-clamp technique was used to record whole-cell and single-channel currents from excised membrane patches. Pressure application of 10 and 100 μM GABA produced inward currents in whole-cell recordings. The amplitude of these currents was voltage-dependent and reversed in sign at the chloride equilibrium potential. Currents in response to prolonged application of GABA declined over time. The rate of decline was more rapid with 100 μM GABA. The decline in current was accompanied by a decrease in the conductance change induced by GABA, suggesting a true desensitization. After 6-8 days in culture, spontaneous inhibitory postsynaptic currents were routinely observed. These currents were chloride-dependent, antagonized by low doses (1 μM) of picrotoxin, and prolonged by pentobarbital (100 μM). Under normal conditions, the decay of these currents did not follow a simple exponential time course but could usually be described by the sum of two exponentials. The role of uptake and channel kinetics in determining the decay of spontaneous synaptic currents is being investigated. Under physiological recording conditions, excised membrane patches were observed to contain numerous voltage-dependent ionic channels. Quiescent patches were obtained by recording in isotonic choline chloride solutions. Under these conditions, exposure of patches to GABA resulted in the appearance of discrete unitary agonist-gated channels. GABA channels in these neurons were nonrectifying over a range of membrane potentials from -60 to +60 mV and had a main conductance state of approx. 20 pS.

These results indicate that cultured chick cerebral neurons utilize GABA as their principal inhibitory neurotransmitter. The lack of glial contamination and the apparent predominance of GABAergic neurons make these cultures a useful model system for study of the molecular mechanism of GABA action. (Supported by USPHS grants NS-11535 and AM-17436.)

249.2 **INHIBITION OF ACETYLCHOLINE RECEPTOR INCORPORATION BLOCKS DEVELOPMENTAL CHANGES IN CHANNEL GATING IN XENOPUS MUSCLE.** P. Brehm, L. Bates*, R. Kream and F. Moody-Corbett. (SPON: S. Fink). Depts. of Physiology & Anesthesiology, Tufts University School of Medicine, Boston, MA, 02111.

Acetylcholine receptors on embryonic Xenopus muscle have been shown, by both noise analysis and single channel recording techniques, to undergo developmental alterations in both channel kinetics and conductance. It was further shown that these changes resulted from an increase in the proportion of a high conductance channel with fast channel kinetics. The increase in the proportion of high conductance channels, when followed in cell cultures prepared from dissociated embryonic myotomal muscle, was paralleled by rapid incorporation of newly synthesized receptors. These findings suggest that receptor incorporation may be required for alterations in channel physiology. To test this hypothesis, we inhibited receptor incorporation by blocking protein synthesis during the time in which changes in channel properties normally occur. Simultaneously, we examined whether there was any effect on the acquisition of adult channel gating properties. Cycloheximide, a blocker of protein synthesis, inhibited greater than 90% of receptor incorporation at a concentration of 50 ug per ml, as measured by $^{125}I$ alpha-bungarotoxin binding to developing Xenopus muscle in vivo. Cycloheximide concentrations below 1 ug per ml were ineffective at blocking incorporation while concentrations exceeding 50 ug per ml were slightly more effective (greater than 95% at 100 ug per ml). Inhibition of receptor incorporation at 50 ug per ml cycloheximide was achieved within two hours of treatment thereby permitting its action on changes in channel physiology to be studied. To examine the effects of inhibiting receptor incorporation on channel properties, we applied 50 ug per ml cycloheximide to half of the cultures prepared from stage 17 to 20 embryos, a time prior to developmental changes in channel gating. The cultures were treated with cycloheximide within 12 hours of plating and recorded from after an additional 24 hours. The extent of changes in gating in both drug treated and nontreated co-cultures was quantitated by measuring the percentage high conductance channel openings. In 4 nontreated cultures the percentage high conductance channels increased from 12% (n=16) to 40% (n=10) over the 24 hour period. These values were not significantly different from previously published values from our lab. In contrast, cycloheximide-treated cultures exhibited only 13% (n=17) high conductance channels after 24 hours, indicating a definitive block of the developmental changes in channel gating. These data indicate an ongoing requirement of protein synthesis for developmental changes in channel physiology. Further experiments are in progress to ascertain whether inhibition of receptor incorporation by blockade at levels other than translation yield similar results. Supported by grants from the NIH (NS18205) and Myasthenia Gravis Foundation.

249.3 PHYSOSTIGMINE REDUCES THE SIZE OF THE FOCAL LESIONS INDUCED BY IRREVERSIBLE ChE INHIBITORS AT THE NEUROMUSCULAR JUNCTION OF RATS: AN ULTRASTRUCTURAL ANALYSIS. M. Kawabuchi*, A.F. Boyne, S.S. Deshpande* and E.X. Albuquerque. Dept. Pharm. & Exp. Ther., Univ. of Maryland Sch. of Med, Baltimore, MD 21201.

Anticholinesterase agents such as physostigmine [Phy, a reversible inhibitor of acetylcholinesterase (AChE)] and sarin (an irreversible inhibitor of AChE) are known to interact with the acetylcholine receptor-ion channel complex. Numerous studies have confirmed that blockade of AChE by a variety of anticholinesterase agents causes a subjunctional myopathy (Ariens et al., 1969; Laskowski et al., 1975, 1977). Pretreatment of rats with atropine (500 µg/kg, subcut.) and Phy (100 µg/kg, subcut.) 30 min prior to the injection of a lethal dose of sarin (130 µg/kg) protected 100% of the animals from lethality (Meshul et al., Neurosci. Abstr. 10:920, 1984; Deshpande et al., 1985, in press). In order to evaluate morphologically this protective effect of Phy, light microscopic and ultrastructural observations were performed on the rat soleus neuromuscular junctions. Samples were taken from rats at 1 hr after sarin (80 µg/kg) treatment, at 1 hr and 24 hr after Phy (100 µg/kg) treatment, and from those pretreated with Phy (100 µg/kg) for 30 min, followed by treatment with sarin (80 µg/kg, a sublethal dose) for 1 hr. All treatment drugs were administered subcutaneously. Sarin-treated muscles showed severe disruption of postjunctional folds, extensive subsynaptic vacuolization of mitochondria and sarcoplasmic reticulum; and severe disorganization and disarrangement of the subjunctional myofibrils. Phy had a selective effect in inducing Z line changes without any gross vacuolization and mitochondrial swelling. Such myopathic changes were largely reversed 24 hr after Phy treatment. Pretreatment of Phy prior to sarin offered marked reduction in the severity of the damage induced by sarin. In most motor endplates, myopathic lesions were recognized, but the banding patterns of the subjunctional myofibrils were preserved. Large vacuoles of mitochondrial origin were seen in the area confined to the endplate sarcoplasm. Statistical analysis of the light microscopic sections revealed that Phy induced a 27% reduction in the average length of lesions and 53% reduction in the average width of lesions. Such effective protection could be related to Phy's ability to penetrate the central nervous system, and/or block ionic channels and decrease channel conduction time in the site of the neuromuscular junction (Supported by U.S. Army Grant DAMD-17-84-C-4219).

249.4 MODIFICATION OF PHOSPHOLIPID FATTY-ACID COMPOSITION AND EFFECTS ON NICOTINIC RECEPTORS IN BC3H-1 CELLS. R.E. Sheridan and R. McGee. Department of Pharmacology, Georgetown University, Schools of Medicine and Dentistry, Washington, DC 20007.

BC3H-1 cells are a continuous muscle cell line which express nicotinic acetylcholine receptors. The potential importance of phospholipid fatty acid composition to the functioning of these receptors was examined by growing cells in media supplemented with specific fatty acids and making patch-clamp recordings of single nicotinic, acetylcholine receptors.

BC3H-1 cells were initially grown in Dulbecco's modified Eagles media (DMEM) supplemented with 20 % fetal bovine serum (FBS) for 2 days. The cells then were transferred to media with 10%or 20% FBS and 32 uM BSA either with or without 128 uM arachidonate (20:4). The cells were allowed to grow to confluency, usually an additional two days, and then were transferred to DMEM with either 2% or 0.5% FBS, exposure to 20:4 being continued as appropriate. Control cells were very deficient in polyunsaturated fatty acids as is normally observed in cells grown with FBS; oleate (18:1 was by far the predominant unsaturated fatty acid. Cells grown with 20:4 were greatly enriched with this polyunsaturated fatty acid and 22:4, its elongation product.

The functional activity of nicotinic receptors was assessed by electrical recordings of single-channel currents. The BC3H-1 cells were transferred to a HEPES-buffered saline solution at 20°C and the patch clamp technique was used to record the activity of individual nicotinic receptors in intact, cell attached, patches of membrane. The patch electrode typically contained saline with 1 to 40 uM carbachol. Cells treated with 20:4 did not show acetylcholine receptor mediated currents, even though paired controls (grown without the 20:4 but with BSA) showed high levels of receptor activity. The membrane patches in the 20:4-treated cells were functional, as revealed by the activity of non-cholinergic channels in the membrane. Acute (one hour) exposure of control cells to 20:4 did not alter the conductance, mean open lifetime, or the voltage-dependence of the mean open lifetime of carbachol-stimulated channels. When the treated cells were removed from the unsaturated fatty acid supplement, there was no recovery of activity after 2 days of growth with 0.5% FBS. However, in cells recovered for 5 days in 2% FBS a high level of nicotinic receptor activity was observed. The properties of these receptors were indistinguishable from those of control cells.

These results suggest that supplementing cells with high levels of polyunsaturated fatty acids may alter synthesis and/or turnover of nicotinic receptors but does not seem to alter the properties of remaining receptors.

249.5 INTERACTIONS OF Ca++ CHANNEL ANTAGONISTS WITH NICOTINIC RECEPTORS. L.Adam* and E.G.Henderson. Dept. of Pharmacology, University of Conn. Health Center, Farmington, Conn. 06032.

We have previously shown that certain organic Ca++ channel blockers have direct effects on the nicotinic receptors of both frog and Torpedo electroplax (N.Y.Acad. Sci., in press.) Of the compounds tested (D-600,Nitrendipine,Bepridil,Nicardipine as well as the partial agonist BAY k-8644) Nicardipine (Nicard, 0.03 to 1 uM) and Bepridil (Bep, 1uM) were the most potent in blocking carbamylcholine (carb, 10 and 100uM) and acetylcholine (Ach)-induced contractures of frog sartorius muscle. Block was time and concentration dependent. Nicard (10uM) did not alter the kinetics of neurally evoked endplate currents (EPC's) or block neurally evoked twitches. It also did not block the depolarization caused by iontophoretically applied Ach.

Nicard and Bep accelerated desensitization which is also both time and concentration dependent. The time course for desensitization was studied using the method of Manthey (J.Gen.Phys. 49:963, 1966) to apply carb (100uM) focally on frog cutaneous pectoris muscles voltage clamped to -90mV. An equation similar to that used for acid and base catalyzed reactions was employed to determine a theoretical relationship between Nicard conc. and the rate of nicotinic receptor desensitization. This equation follows from the theoretical basis that the nicotinic receptor bound by Nicard is responsible for the acceleration of desensitization independent of whether the receptor-ionophore complex is in its open, closed, or desensitized conformation. The following equation was employed:

$$k=k^{o}+k_{cat}\,(N/N+K_{d}),$$

where k and $k^{o}$=rate constants in the presence and absence (with all other factors affecting desensitization remaining constant) of Nicard at a conc. of N; $K_d$=diss. constant and $k_{cat}$ is a catalytic coefficient which remains constant as a function of N. Using this equation, an electrophysiological $K_d$=0.61µM for Nicard was determined. When the antagonism by Nicard of $^3$H-PCP binding to Torpedo microsacs was studied, a biochemically measured $K_d$=0.87uM was obtained. These results are significant in describing a $K_d$ using frog muscle in a manner that does not require a second biological system (i.e. Torpedo.) Also, Nicard allows a broad range of concentrations for desensitization studies to be used that do not affect any aspect of the kinetics of EPC's. Nicard has a $K_d$=0.61 uM and does not affect EPC's at 10uM; SKF 525-A, a prototype desensitizing agent alters the kinetics of EPC's at conc. of 2-5uM.

Although Nicard antagonizes PCP binding to microsac membranes, in electrophysiological experiments it enhanced the effects of PCP in altering EPC decay and peak amplitude. Bep, which is 10 fold more potent in antagonizing PCP binding, did not enhance the effect of PCP on EPC's. Studies are in progress to assess the mechanism whereby Nicard and Bep increase the rate of desensitization.

249.6 OPEN-CHANNEL BLOCKADE BY CHLORISONDAMINE (CHL) LEADS TO A STABLE NON-CONDUCTING STATE OF THE ACETYLCHOLINE (ACh) CHANNEL AT THE FROG END-PLATE. A. Neely*, C.J. Lingle (SPON:P.Cancalon). Dept. Biol. Sci., Florida State Univ. Tallahassee, FL 32306.

The effect of the long-lasting ganglionic blocker, CHL, on end-plate currents (EPCs) was studied at the neuromuscular junction (NMJ) of the cutaneous pectoris in the grass frog, Rana pipiens, using conventional two microelectrode voltage-clamp. First, effects were examined on miniature end-plate currents (MEPCs). The major effect of CHL (concentrations 1-10 M) on MEPCs is to shorten the decay time. Changes in amplitude are noticed after prolonged application or high concentrations. The decay is fully described by a single exponential relaxation process in controls and in the presence of CHL. The inverse of the time constant increases linearly with drug concentration, with a slope that increases with hyperpolarization. This observation is consistent with the mechanism of open-channel blockers which are able to plug the pathway for ion flow once the channel is opened following the binding of two ACh molecules (Peper et al, 1982; Physiol. Rev. 62:1271). The presence of a single exponential decay is due to the lack of significant recovery in the time scale of a single MEPCs. Assuming this model, the estimate for the forward blocking rate is $10^{7}$-$10^{8}$ $M^{-1}sec^{-1}$ with a voltage-dependence of 30 mV/e-fold.

Unblocking kinetics were examined with EPCs evoked by iontophoretic application of agonist. Double pulse experiments reveal a slow unblocking process that is agonist-independent suggesting that the channel remains open while blocked. Such slow channel blockade is usually explained by steric interference with the gating mechanism. However, experiments with repetitive iontophoretic pulses reveal an additional fraction of blockade that persists after washout of CHL, despite the subsequent agonist application. Previous studies in the lobster NMJ (Lingle,1983; J.Physiol.339:395) show that full recovery from a similar stable blockade can be obtained only with agonist application suggesting that channel closure occurs with the blocker trapped inside. A similar model may be applied to the frog NMJ with the modification that, following channel closure, the channel is essentially constrained from reopening by the presence of CHL in the ion flow pathway. ( Supported by NIH NS-19139 and MDA grant to CJL ).

249.7 POSTSYNAPTIC POTENTIATION IN FROG NEUROMUSCULAR JUNCTION. E. Kus* and M.I. Glavinovic (SPON: B. Esplin), Departments of Anaesthesia Research and Physiology, McGill University, Montreal, Quebec, Canada H3G 1Y6.

During sustained application of an agonist in a neuromuscular junction there is a progressive loss of response of receptors to it due to desensitization. At the beginning of the steady ACh application prior to desensitization however, the test responses are enhanced due to post-synaptic potentiation (Katz & Thesleff, 1957, J. Physiol. 138, 63-80). Postsynaptic potentiation (PSP) was shown to be particularly pronounced at low temperatures and with cholinesterase inhibited where it was attributed to accumulation of monoliganded presumably non-conducting ACh receptor complexes (Feltz & Trautmann, 1980, J. Physiol. 299, 533-522). The present study is a further attempt to examine how PSP changes with steady ACh application that produces clear desensitization.

The experiments were done on the frog (Rana pipiens) cutaneous pectoris preparation at room temperature (19-20 °C) in physiological Ringer using voltage clamp technique. The end-plates were localized using a (Zeiss) Nomarsi water immersion microscope (x320), and acetylcholine was applied iontophoretically.

Iontophoretic pulses were applied at a frequency of 5 Hz for 5 sec, followed by 15 sec of no application and were chosen to produce initial responses of less than half maximal value (25-45%). This was repeated every 20 seconds, 10-20 times. During an initial short iontophoretic train, amplitudes of the resulting ACh responses first increase and then decrease, and are followed by recovery in between iontophoretic trains. This pattern of response remains throughout ACh application.

PSP (defined as the fractional increase in the ACh response at the beginning of a short iontophoretic train) increases with ACh application between 30 and 145% although ACh responses are reduced to usually less than 20% of their initial value. Essentially the same answer is obtained if desensitization is produced by repeated iontophoretic applications of ACh pulses and PSP tested by inserting ACh pulses at regular intervals.

The increase in PSP is unlikely to be explained by accumulation of monoliganded ACh receptor complexes. The presence of cholinesterase ought to prevent such accumulation. PSP probably increases because desensitized receptors accumulate. Because of their high affinity for ACh, they make the dose response curve progressively more sigmoidal and PSP more evident.

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249.8 **INHIBITION OF ABNORMAL ELECTRICAL ACTIVITY WITH ELECTRICAL STIMULATION IN-VITRO.** **D. Durand, Applied Neural control Laboratory, Dept. of Biomedical Engineering, Case Western Reserve University, Cleveland, OH 44106**

**Electrical stimulation of the peripheral nerves and muscles has proven very successful in restoring partial motor function in patients suffering from various motor disorders. However, the clinical applications of Functional Electrical Stimulation (FES) in the CNS have fallen short of expectations. A particularly difficult problem is to generate neuronal inhibition using electrical stimulation. Recent studies have demonstrated the role of electrical fields in the synchronization of epileptic discharges, suggesting a possible solution. This study was then designed to investigate the possibility that electrical stimulation could in turn be used to generate inhibition of epileptic-like seizures.**

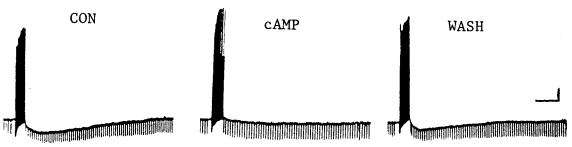
**Transverse hippocampal slices were prepared from Sprague-Dawley rats (150-200g). In order to generate repetitive epileptic activity, 7000 units of penicillin or 200uM of picrotoxin were added to the artificial CSF. Orthodromic responses with 3 to 10 population spikes were triggered with a stimulating electrode in the stratum radiatum and recorded with a glass microelectrode (5 MOhm) located in the CA1 cell layer. A 50um diameter stainless-steel electrode located close to the recording electrode was then activated with a current generator delivering pulses of various amplitude and width. The current pulse was applied following orthodromic stimulation with a variable delay.**

**In all 32 slices that were tested, the application of the current pulse generated a large amount of inhibition (reduction of the amplitude of all the population spikes following the current pulse). The inhibition was directly related to the amount of charge injected. The maximum amount of inhibition (91+4%) was obtained with 94+13nC (N=22) when the stimulation pulse was positive (anodic) and with only 24+4 (N=10) with a negative pulse (cathodic). The time of application of the pulse was the most crucial parameter and was restricted to a time window of 0.5 ms centered on the positive peak preceding a population spike. If applied outside the window, the current pulse could increase instead of decrease the neuronal activity.**

**The mechanisms of the inhibition are not yet clear, but probably involve an intracellular effect inhibiting action potentials and an extracellular "desynchronizing" effect. Both effects are under study with intracellular recording techniques and Current Source Density Analysis methods.**

249.9 REPETITIVE STIMULATION PRODUCES A VERY LONG AFTERHYPERPOLARIZATION IN RAT HIPPOCAMPAL PYRAMIDAL CELLS IN VITRO. A. Williamson* and B.E. Alger (SPON: D.S. Ruchkin). Dept. of Physiol., Univ. of MD. Sch. Med. Baltimore, MD, 21201.

An epileptic seizure is a complex electrophysiological event involving periods of repetitive neuronal firing which can last tens of seconds. We have studied mechanisms which may participate in terminating seizures in the rat hippocampal slice using prolonged (10 Hz, 5 sec.) synaptic stimulation to mimic a seizure. Intracellular recordings from 50% of the cells showed that this stimulation produced a large depolarization during the train followed by a very long hyperpolarization (VLH) which can last 0.5 to 3 minutes at 30°C. The VLH has a peak amplitude of 3-15 mV and an associated conductance increase of 10-50%. We hypothesized that the VLH could be due to an increase in an ionic conductance or to activation of the sodium/potassium pump. The pump may be partially involved since dihydrooubain, a specific sodium/potassium pump inhibitor, reduced the VLH in several cells. The VLH cannot, however, be totally attributed to an electrogenic pump effect. The evidence suggests that a potassium conductance is involved. First, 5 mM cesium chloride blocked the VLH in four out of five cases. Second, the VLH had a null potential of -78 mV in control saline (5.4 mM potassium) which shifted to -69 mV in saline containing 8.1 mM potassium. This value approaches the potential reported for the potassium equilibrium potential in these cells. Third, cyclic AMP, which blocks a calcium-dependent potassium afterhyperpolarization (AHP) evoked by a brief spike train in the hippocampus, significantly reduced the VLH. (See Fig 1. Calibration: 5 mV and 10 sec.) The VLH may differ from the AHP, however, since carbachol, which also blocks the AHP, reduced the VLH duration and increased or had no effect on either the VLH amplitude or conductance. Our data suggest that a very long-lasting calcium-dependent potassium potential is activated by repetitive synaptic stimulation and may, therefore, play a role in seizure termination.




249.10 VOLTAGE-CLAMP ANALYSIS OF A SYNAPTICALLY ACTIVATED LATE HYPERPOLARIZATION IN HIPPOCAMPAL CA3 NEURONS. R. H. Thalmann and J. J. Hablitz. Depts. of Cell Biology and Neurology and Program in Neuroscience, Baylor Col. of Med., Houston, TX 77030.

Activation of the mossy fiber input to hippocampal CA3 neurons, at levels that are subthreshold for action potential (AP) generation, produces two successive hyperpolarizing potentials. The early component is chloride mediated, whereas the late hyperpolarization (LH) is thought to represent an increase in potassium conductance. The amplitude of the LH is linearly related to membrane potential over the range -60 to -120 mV (Thalmann, R. H., Neurosci. Lett. 46:103). Using current- and voltage-clamp techniques, we investigated the nature of the conductance change underlying the LH.

Intracellular recordings were obtained from CA3 neurons of rat hippocampal slices maintained in vitro. Synaptic potentials were elicited via bipolar stimulation of the mossy fiber pathway. Using current-clamp techniques, the conductance underlying the LH was estimated from the change in the voltage deflection produced by a train of hyperpolarizing current pulses. At the peak of the response (approx. 140 msec after the stimulus), the average conductance increase was 15.3 ± 6.2 nS, while the half-decay time of the response was 120 ± 23 msec. These values were relatively constant over the voltage range -55 to -110 mV. The reversal potential for the LH was between -90 and -95 mV. Under voltage-clamp conditions, using a single-electrode time-share circuit, two currents were observed, corresponding to the early and late hyperpolarizing potentials. At a holding potential of -55 mV, an early, large outward current was followed by a less prominent slow outward current. The initial current generally peaked in 10-12 msec, reversed at -75 mV, and had a slope conductance of approx. 80 nS. The second current peaked at approx. 120 nS and reversed near -95 mV. Plots of current amplitude versus membrane potential were linear, and linear regression lines fitted to the data indicated a conductance of 12.3 ± 4 nS. The decay of this current, in contrast to that of the early response, was not demonstrably voltage dependent over the range studied.

These results suggest that the synaptically evoked early and late hyperpolarizing potentials arise from different ionic mechanisms. The consistency of the conductance underlying the LH, regardless of whether or not APs or voltage transients occurred in the impaled cell, indicates that the late hyperpolarization arises, at least in part, as a consequence of activation of transmitter- rather than voltage-gated channels. The potassium conductance that presumably underlies the LH appears to differ from several previously described voltage-gated potassium conductances in its lack of rectification and voltage sensitivity of decay. (Supported by USPHS grants NS-21713 and AA-06077.)

249.11 INTRA- AND EXTRACELLULAR ANALYSIS OF SPONTANEOUS ACTIVITY IN HIPPOCAMPAL NEURONS IN CULTURE. A.T. Malouf and D.L. Gruol. Div. of Preclin. Neurosci. and Endocrin., Scripps Clinic and Research Foundation, La Jolla, CA 92037

Extracellular unit recordings from rat hippocampal neurons (HPNs) derived from fetal tissue and maintained in culture for several weeks reveal that these neurons exhibit at least three patterns of spontaneous electrical activity (Malouf and Gruol, Neurosci. Absts, 1984). We have now used intracellular recordings to identify the mechanisms generating the spontaneously activity and to characterize the development of these mechanisms during HPN maturation in culture. Intracellular recordings, in agreement with extracellular observations, demonstrated that the expression of spontaneous electrical activity develops slowly over the first two weeks in culture. During this time, action potentials (AP's) or post synaptic potentials (PSP's) are rarely recorded. The reasons for this low spontaneous activity are not presently known, but the HPNs are capable of producing APs in response to either depolarizing current injections or application of glutamate. Furthermore, repetitive depolarizations which elicit AP's result in the influx of large numbers of PSP's, presumably through recurrent pathways, indicating that these cells are synaptically coupled by the second week in culture. One possible explanation is that expression of spontaneous activity depends on the development of endogenously generated activity, such as the type attributed to CA3 HPNs, which in turn excites the other HPNs. The existence and temporal development of endogenous activity in the cultured HPNs is currently under investigation. Extracellular recordings demonstrated that by the third week in culture most cells exhibit some form of spontaneous activity. While a few cells fire in a random pattern of single spikes, greater than 80% of the HPNs display a bursting type of firing pattern. The burst patterns can be divided into two types. One type is a simple burst pattern consisting of repetitive clusters of 3-8 single spikes. The second type is characterized by prolonged bursts (20-50 spikes) comprised of smaller (2-6 spike) bursts. Intracellular recording revealed a fundamental difference between the two bursting patterns. While spikes are usually associated with a volley of PSP's in both patterns, spikes are elicited from PSP's which rise and return to the baseline after each spike in the simple pattern. In the complex pattern, however, bursts of spikes ride atop a general membrane depolarization of 2-5 mV. These data suggest that the two bursting patterns may result from different synaptic and/or endogenous mechanisms. Identification and analysis of the mechanisms which generate these patterns should aid in our understanding of the cellular and synaptic physiology of HPNs. (Supported by NIAAA 06420 and 07456)

249.12 CAFFEINE-INDUCED CHANGES IN TTX SENSITIVE SLOW DEPOLARIZATIONS IN CA1 PYRAMIDAL NEURONS. K. Baker* and B.A. MacVicar, Dept. of Medical Physiology, University of Calgary, Calgary, Alberta T2N 4N1, Canada.

Intracellular calcium is implicated in regulating many cellular properties. Caffeine induces intracellular calcium release in neurons and elicits a slow depolarization. We have examined the effects of caffeine on slow depolarizations in hippocampal neurons which may be controlled by the release of intracellular calcium.

Intracellular recordings were obtained from CA1 pyramidal neurons in rat hippocampal slices with microelectrodes (100-120 mΩ) filled with 2 M K acetate. Brain slices were maintained at 33° and were superfused at 1 ml/min. Extracellular application of caffeine (5 mM) in normal saline caused epileptiform bursting in CA1 pyramidal neurons. This action could result from increased synaptic transmission and enhanced calcium spiking. We therefore examined caffeine's actions when intracellular calcium release would be the only effect, ie. after blocking synaptic transmission and calcium spiking. Cells were impaled in control solutions initially, then solutions containing low calcium (0.1 mM) and cadmium (2 mM) were perfused. This was observed to block synaptic transmission and calcium spikes. After the recordings were stable in cadmium, caffeine was perfused and in most cells there was a 10-20 mV slow depolarization often with superimposed rhythmic depolarizations. This slow depolarization did not occur in low sodium solutions. The threshold was determined by observing the onset of the slow depolarization during a ramp current injection. After the threshold was observed to be stable in low calcium-cadmium solution, caffeine was introduced. In five stable recordings following the addition of caffeine (5 mM) the cells depolarized 11.6 mV from 69 ± 1.4 mV (± S.D.) to 57.4 ± 2.9 mV and the threshold for the slow depolarization changed from 66.2 ± 1.9 mV to 75.4 ± 1.7 mV. In all five cells the slow depolarization was subsequently blocked by TTX (1 mM). Therefore caffeine effectively reduced the threshold for the TTX sensitive slow depolarization. This action could be the result of intracellular calcium release which suggests that the slow inward $Na^+$ current may be regulated by intracellular calcium. Alternatively the caffeine could directly effect the slow inward current.

Supported by Alberta Heritage Foundation for Medical Research and MRC of Canada.

249.13 EXCITATORY EFFECTS OF DELTA-9-TETRAHYDROCANNABINOL ON HIPPOCAMPAL NEURONES RECORDED IN VITRO S.A. Deadwyler R.E.Hampson and G.A. Marlow. (Spon:S.Stwertka) Bowman Gray School of Medicine, Winston-Salem, N.C. 27103.

Previous extracellular studies of the effects delta-9-Tetrahydrocannabinol (THC) on the hippocampus have suggested a biphasic dose response relationship. Extremely low doses of this compound were found to increase the excitability of CA1 cells in the hippocampal slice, while higher doses decreased excitability (Foy et. al. Brain Res Bull 1982). Recent studies in cat spinal motorneurones have indicated that delta-9-THC increases excitability by enhancing EPSPs and decreasing IPSPs. These changes were associated with a concomitant increase in membrane resistance (Turkanis and Karler Brain Res 1983). We have investigated whether or not similar cellular and synaptic changes are responsible for the reported effects of delta-9-THC in the hippocampus.

Intracellular recordings were made from 44 neurones in the CA1 region of the rat hippocampal slice preparation. Slices were maintained in two different types of incubation chambers and drug delivery was by three different techniques in order to maximize the capability of discerning the direct effects of delta-9-THC: microdrop application of THC ($10^{-4}$M) to the surface of the slice in a static chamber ; superfusion with THC ($10^{-7}$ to $10^{-4}$M); or bolus injections of THC ($9.5x10^{-3}$M) into the line of a flow chamber. THC was dispersed by sonification and administered in a suspension of pluronic F68 detergent (concentration in medium 0.18-0.56mM). The two prominent effects of THC application at all dose levels was an increase in excitability as indicated by 1) membrane depolarization and induced or increased spontaneous firing, and 2) increased discharge to intracellular depolarizing current pulses (60-70% of cells tested). In addition these changes were accompanied by an increase in firing threshold. Membrane resistance was increased in those cells which showed increased spontaneous discharge. The Schaffer collateral elicited EPSP-IPSP sequence was differentially affected by low vs high doses of THC. In 20% of the cells tested application of THC resulted in no change or a decrease in excitability. Many of the cellular and synaptic effects of THC were duplicated by application of carbachol (80 uM) to the bathing medium of cells which recovered from prior exposure to THC (Cole and Nicoll Brain Res. 1984). The results confirm the findings of previous investigations by demonstrating that delta-9-THC produces excitatory changes in CNS neurones.

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249.14 **INHIBITORY POTENTIALS DECREASE PREFERENTIALLY INWARD RECTIFYING CURRENTS IN RAT AND HUMAN CORTICAL NEURONS MAINTAINED "IN VITRO"** V.Tancredi*,M.Avoli and N.Agopyan* (SPON: S.Gauthier). Montreal Neurological Institute and Department of Neurology & Neurosurgery, McGill University, Montréal, Québec, H3A 2B4, Canada.

In several different neuronal systems (e.g. motoneurons) the inhibitory action of post-synaptic potentials derive from a combination of hyperpolarization and shunting of EPSPs. We analyzed further this aspect in cortical neurons in slices of the rat hippocampus and somatosensory region. Similar experiments were also carried out in neurons in slices of "normal" (i.e. non-epileptic) neocortex (2nd temporal gyrus) taken during surgery for brain tumor. Intracellular (presumably intrasomatic) recordings were performed with K-acetate or KCl filled pipettes placed in the stratum pyramidale of the CA1 subfield, or deep layers (5th, upper 6th) of the rat or human neocortex. Extracellular focal stimulation was achieved with sharpened monopolar tungsten electrodes positioned in the stratum radiatum of hippocampal slices and in the underlying white matter or within the layers of neocortical slices.

Stimulus induced responses in recordings performed with Kacetate filled microelectrodes were composed of an EPSP-IPSP succession in the hippocampus and of a mainly depolarizing potential in rat and human neocortex. In the latter cases, however, an IPSP was disclosed by decreasing the resting membrane potential (Vm) by 5-10mV. Also, when KCl microelectrodes were employed this component of the stimulus induced response was sensitive to $Cl^-$ injections. Both the hippocampal and the neocortical IPSPs were capable of reducing and abolishing the occurrence of action potentials evoked by depolarizing intracellular pulses, an effect which resulted from the hyperpolarizing trend (when present) and the increase in membrane conductance (gm). Thus, when hyperpolarizing and subthreshold depolarizing intracellular pulses were injected before and after the stimulus, a large increase in gm was associated with the IPSP. The gm increase was greater for voltage responses in the depolarizing direction than those in the hyperpolarizing one. This feature was: (i) observed in rat hippocampal and neocortical cells as well as human neocortical neurons; (ii) present in both neurons recorded with KCl and K-acetate microelectrodes and (iii) detected in the latter cases even at a Vm close to the equilibrium potential for the hyperpolarization.

These finding suggest that cortical IPSPs affect preferentially (voltage dependent) inward currents which are particularly pronounced in both rat and human cortical "in vitro" neurons. Also such a mechanism might shunt EPSPs without decreasing to the same extent summating hyperpolarizing potentials.

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249.15 AN IN VITRO BRAIN SLICE PREPARATION TO STUDY CHOLINERGIC NUCLEI OF THE BASAL FOREBRAIN (Ch1-Ch4). W.H. Griffith and R.T. Matthews. Departments of Medical Pharmacol. and Toxicol. and Medical Anatomy, College of Medicine, Texas A&M Univ., College Station, TX 77843

An in vitro brain slice preparation from guinea pigs and rats has been developed to study the anatomy, physiology and pharmacology of neurons within specific nuclei of the basal forebrain. These nuclei include the medial septum (Ch1), vertical limb of the diagonal band (Ch2), horizontal limb of the diagonal band (Ch3) and the nucleus basalis (Ch4). These nuclei contain both cholinergic and non-cholinergic cell bodies that send projections to the neocortex and hippocampus. Degeneration of neurons within these nuclei have been implicated in progressive dementias, including senile dementia of the Alzheimer's type (SDAT).

Coronal serial sections of the forebrain (400-500 µm) were cut using a Vibratome tissue slicer. Slices were transferred to a holding chamber, warmed (34-35°C) and oxygenated with 95% $O_2$, 5% $CO_2$. A single slice was later transferred to a recording chamber, held completely submerged and continually perfused with a physiological solution. Intracellular and extracellular recordings were made from neurons in all four nuclei. Based on extracellularly recorded spontaneous firing patterns in the Ch1-Ch3 areas, two populations of cells were observed, either burst-firing (40%) or rhythmically-firing cells (60%, N=20). Both cell types were excited by bath application of bethanechol or carbachol ($10^{-6}$-$10^{-4}$ M, 66% of the cells responding, N=6). Intracellular recordings also revealed spontaneous activity, including both epsps and action potentials. However, the burst-firing pattern was not observed intracellularly (N=25). Neurons in the Ch1-Ch2 region exhibited stable resting membrane potentials (-60 to -80 mV), high input resistances (50-200 MΩ) and fast (~2 ms duration) TTX-sensitive action potentials (70-100 mV). One significant difference between cells was the duration of the post-spike afterhyperpolarization (AHP). Both long duration ( ~200 ms) and short duration (<20 ms) AHPs could be recorded in different cell types. Orthodromic epsps, and action potentials could be recorded in Ch2/Ch3 cells following focal stimulation close to the recording site or placement of the bipolar stimulatory electrode some distance away in the medial septum, suggesting the presence of intact synaptic circuits between Ch1, Ch2 and Ch3 in this slice preparation. In summary, preliminary electrophysiological identification of these neurons suggests more than one cell type may be present in these nuclei. Future experiments will be directed towards extending this characterization to cholinergic and non-cholinergic neurons.
(Supported by NIH Grant AG05282 and Biomedical Research Support Grants.)

## POSTSYNAPTIC MECHANISMS III

250.1 PHORBOL ESTERS BLOCK THE RESPONSE OF RAT HIPPOCAMPAL PYRAMIDAL NEURONS TO BACLOFEN. B. E. Alger, J. M. Baraban and S. H. Snyder, Dept. Physiol. Univ. MD. Sch. Med., Baltimore, MD. 21201 and Dept. Neurosci., Johns Hopkins Univ. Sch. Med., Baltimore, MD. 21205.

We have recently reported that bath-application of active phorbol esters selectively blocks a component of the synaptically evoked potential recorded in rat hippocampal pyramidal cells in the slice preparation (Proc. Natl. Acad. Sci., USA, 82:2538, 1985). This component, the late hyperpolarizing potential (LHP), appears to be a potassium-dependent synaptic potential, however the neurotransmitter responsible has not yet been identified. If the phorbol ester-induced antagonism of the LHP is a postsynaptic effect, then phorbol esters can be used to screen potential neurotransmitter candidates. Recent suggestions that the LHP might be mediated by the bicuculline-insensitive $GABA_B$ receptor, prompted us to begin this screening by examining the response to baclofen, a specific agonist of this receptor.

Intracellular recordings were made from hippocampal pyramidal cells in vitro and the LHP and responses to bath-applied baclofen (25 - 100 uM for 30 - 60 seconds) studied. Under these conditions baclofen produces a 5-10 mV hyperpolarization which is associated with a 15 - 35 % decrease in input resistance. Full recovery occurs in 10 minutes.

Following recording of responses in the control saline, a saline containing phorbol 12, 13 diacetate (PDA), 1 uM, was perfused. Baclofen (plus PDA) was reapplied at intervals. PDA consistently reduces the hyperpolarization and conductance increase of the baclofen response 50-90%, depending upon the dose involved and duration of application. Both effects are reversible. The block of the LHP can also be attributed to a block of its conductance mechanism and not to a shift in its reversal potential. Maximal antagonism of the baclofen response is not seen until the depressant effect of PDA on the LHP is well-established; roughly 20-30 minutes after PDA is first applied. As previously reported, phorbol esters depolarize the neuronal membrane slightly (3- 8 mV) and cause an increase (≤20 %) in input resistance. Accordingly we tested the effects of baclofen both at the new "resting" potential, and after cells were repolarized to the original resting potential with current injection. The block was seen under both conditions.

Our data are not incompatible with the hypothesis that GABA, acting at the $GABA_B$ receptor, is the LHP transmitter, however the specificity of the phorbol ester antagonism for baclofen, as against other transmitter candidates, is still under investigation.

250.2 GUANINE NUCLEOTIDES STIMULATE PHOSPHOLIPASE C HYDROLYSIS OF PHOSPHATIDYLINOSITOL 4,5-BISPHOSPHATE IN RAT CORTICAL MEMBRANES. R.A. Gonzales and F.T. Crews. Dept. of Pharmacology, University of Florida Medical School, Gainesville, FL 32610.

Inositol phospholipid hydrolysis has been suggested to be the initial step in a signal cascade-amplifier system for many neurotransmitters in the CNS. Agonist stimulation of the receptor activates a phospholipase C which hydrolyzes membrane inositides and leads to the production of diacylglycerol and inositol phosphates. We have investigated the possible involvement of guanine nucleotides as part of the transduction system from agonist-receptor complex to activated phospholipase C. Rat cerebral cortical membranes were prepared from slices which had been incubated with [$^3$H]inositol to label inositides. Slices were homogenized gently with a glass-glass homogenizer in Krebs-Ringer-EDTA buffer and the homogenate was centrifuged at 1000xg for 20 min. The resulting pellet was then resuspended in 20 mM Tris, repelleted, and washed again with Tris. Gpp(NH)p addition to membranes stimulated the production of [$^3$H]inositol phosphates from the labelled membranes. The $ED_{50}$ was approximately 30 µM with a maximal effect at 100 µM. A time course study indicated that the reaction was maximal at a time of 2 min. Separation of the individual inositol phosphates which were formed by stimulation with 100 µM Gpp(NH)p showed that inositol trisphosphate formation was increased by 30-fold. Inositol bisphosphate and inositol 1-phosphate were also increased. Gpp(NH)p did not stimulate the hydrolysis of [$^3$H]phosphorylcholine from [$^3$H]phosphatidylcholine. The Gpp(NH)p effect on inositide hydrolysis was not dependent upon $Ca^{2+}$ except at concentrations above 100 µM. $Ca^{2+}$ alone stimulated [$^3$H]inositol phosphate production up to 100 µM. Higher calcium concentration inhibited the response. $Mg^{2+}$ by itself inhibited the nonstimulated production of inositol phosphates but had no effect on the Gpp(NH)p response. GTP, GDP, and GMP had smaller stimulatory effects than Gpp(NH)p. The lack of effect of App(NH)p and dibutryl cAMP suggests that the stimulation of phospholipase C hydrolysis of inositides is specific for guanine nucleotides. Previous studies have characterized a $Ca^{2+}$ dependent phosphoinositide phosphodiesterase. Our finding that Gpp(NH)p stimulated hydrolysis is not calcium dependent suggests the presence of another enzyme or that guanine nucleotides alter the calicum dependence of this phosphodiesterase. In any case, these results suggest that phosphatidylinositol 4,5-bisphosphate hydrolysis by a phospholipase C is specifically stimulated by guanine nucleotides and that a guanine nucleotide binding site may be involved in receptor stimulated inositide hydrolysis in brain. (Supported by NIAAA AA06069).

250.3 PHOSPHOPROTEIN REGULATION BY CYCLIC AMP AND BY PHORBOL ESTERS IN CORTICOTROPH TUMOR CELLS. J.F. Bishop, J.M. Farah, Jr., J. Patel, and T.L. O'Donohue. Experimental Therapeutics Branch, NINCDS, and Neurochemistry Branch, NIMH, NIH, Bethesda, MD 20205.

The role of protein phosphorylation in the regulation of cellular functions such as the synthesis, processing and secretion of biologically active proteins and peptides is a major current research focus. Although the mechanisms are unclear, it has been suggested that specific phosphoproteins are involved in neurohormone release and in the regulation of neurohormone gene expression. Stimulation of anterior pituitary corticotroph tumor cells (AtT-20/D16-16) with analogues of cyclic adenosine monophosphate (cAMP) or with 12-O-tetradecanoyl-phorbol-13-acetate (TPA) results in the dose-dependent release of pro-opiomelanocortin (POMC)-derived peptides (ACTH, β-lipotropin, β-endorphin). Since cAMP and TPA exert their pharmacological actions by activating different protein kinase systems--the cAMP dependent protein kinase (PKA) and the protein kinase C (PKC) systems, respectively--neurohormone release and precursor synthesis may be regulated by common or by different phosphoproteins. The present experiments were designed to identify and compare protein kinase substrates responsive to cAMP and toTPA in the cytosolic fraction of AtT-20 cells. AtT-20 cells were loaded with $^{32}$P-orthophosphate in a phosphate-free buffer, rinsed, and dibutyryl cAMP (1 mM), TPA (150 nM) or control solutions were added. After a five minute incubation period, the cells were homogenized and the cytosolic fractions were collected following a low speed centrifugation step. TCA precipitable proteins were then analyzed by two dimensional gel electrophoresis and quantitated by densitometry.

Stimulation of AtT-20 cells with dibutyryl cAMP resulted in a moderate increase in $^{32}$P-incorporation by an approximately 87 kd protein with a pI of about 4.8-5.0, as well as a moderate decrease in $^{32}$P-incorporation by a pair of proteins of approximately 19 kd with pIs of about 6.6-6.8. In response to stimulation with TPA, several cytosolic proteins exhibited increases in $^{32}$P-incorporation including profound increases in a 39 kd protein with a pI of about 5.0 and profound increases in the same pair of 19 kd proteins that were dephosphorylated by dibutyryl cAMP stimulation. In addition, the 87 kd protein that was moderately phosphorylated in response to dibutyryl cAMP was profoundly phosphorylated by TPA. Prominent decreases in $^{32}$P-incorporation induced by TPA were exhibited by two proteins of about 33 and 67 kd, with pIs of about 4.6-4.8. The results of these experiments demonstrate that activation of second messenger systems linked to the PKA and the PKC systems can induce alterations in the phosphorylation states of both distinct and shared phosphoproteins.

250.4 PROTEIN PHOSPHORYLATION BY PROTEIN KINASE C IN THE CELL-FREE SYSTEM OF RAT AORTA. T. Nakaki*, B.C. Wise, D.-M. Chuang and E. Costa (SPON: J.L. Meek). Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hospital, Washington, D.C. 20032.

In rat aorta, 5-HT elicits smooth muscle contraction through activation of 5-HT$_2$ receptors. We reported that the 5-HT$_2$ receptors differentially regulate the activity of a voltage-dependent $Ca^{2+}$ channel and phospholipase C in the process of rat aorta contraction. Moreover, we suggested that diacylglycerol, a metabolite of phosphoinositide which activates protein kinase C might play an important role in mediating tonic component of the 5-HT-induced contraction of rat aorta. To test this hypothesis, we carried out the phosphorylation study if there are substrate proteins for protein kinase C in rat aorta.

Male rats were decapitated and 3 cm segments of thoracic aorta were excised, adhered fat and connective tissue were removed and the aortic segments were incubated for 1 hr at 37$^0$C in a calcium-free Krebs Ringer bicarbonate buffer. The tunica intima was removed by scraping several times with a razor blade. The supernatnat after centrifugation at 100,000 x g was used for the phosphorylation study. The reactions were carried out at 30$^0$C for varying times and were terminated by the addition of sodium dodecyl sulfate (SDS)-stop solution The samples were subjected to SDS-polyacrylamide gel electrophoresis and autoradiography.

Addition of 1 mM $Ca^{2+}$ caused an increase in phosphorylation of several proteins. These include 16-, 20-, 36-, 45-, 48-, 60-, 92.5-kilodalton proteins. Among these proteins, the addition of phosphatidylserine plus $Ca^{2+}$ further increased the phosphorylation of proteins of molecular weight (in kilodalton), 16, 20 and 92.5. In the absence of $Ca^{2+}$, phosphatidylserine failed to increase protein phosphorylation.

Slight differences in a time course of phosphorylation of those proteins were noted. Net increases in phosphorylation of 20 and 92.5 kilodalton is the most evident at 20 sec, while that of 16 kilodalton was the most obvious at 60 sec.

We have also examined the effects of 12-O-tetradecanoylphorbbol-13-acetate (TPA) and 1,2-diolein which are activators of protein kinase C and the biologically inactive analogue 4 -phorbol-12,13-didecanoate (4 PDD). TPA and 1,2-diolein, but not 4 PDD, increased protein phosphorylaton of molecular weight 20, 88 and 92.5 kilodaltons.

In conclusion, consistent with our previous hypothesis, in rat aorta we could identify substrate proteins for protein kinase C, which might be activated after 5-HT$_2$ receptor stmulation.

250.5 INOSITOL TRISPHOSPHATE INJECTIONS AND IMAGING OF THE CALCIUM INDICATOR FURA-2 IN MOLLUSCAN NEURONS. L.Fink*, J.A.Connor, J.A.Strong and L.K.Kaczmarek. Depts. Pharmacology and Physiology, Yale Univ. Sch. of Med., New Haven, CT 06510 and Bell Laboratories, Murray Hill, NJ 07974.

Inositol trisphosphate (IP3) has been proposed as a second messenger that controls the release of intracellular calcium ions in a variety of cell types. We have begun to investigate the effects of IP3 on the electrical properties of the peptidergic bag cell neurons of Aplysia. Bag cell neurons in primary culture were impaled with two microelectrodes, one containing inositol trisphosphate solution (0.8 mM in 0.6M KCl) and the other containing 3M KCl for voltage recording. Brief pressure injection of IP3 into bag cell neurons reproducibly hyperpolarized the membrane potential for approximately 60 seconds. This was accompanied by a 20% reduction in input resistance that recovered with the same time course as the membrane potential. The peak amplitude of the IP3 induced hyperpolarizations was 20 to 30 mV. IP3 injection was also observed to reduce the amplitude of action potentials evoked by depolarizing currents, recovery taking place within one minute of injection. Control injections of either KCl or myo-inositol had no effect on membrane potential, input resistance, or size of action potentials.

Because these effects of IP3 are consistent with the activation of a calcium dependent potassium current, we have begun to measure changes in intracellular calcium ion concentrations in these isolated neurons. Bag cell neurons, growing on #1 cover slips, were labeled in a loading solution containing 5uM fura-2 for 1 hr. They were then incubated in artificial seawater at 26 to 28 °C for at least 2 hrs in order to de-esterify the indicator. Fluorescence (475-525 nm) of single bag cell neurons was measured with a CCD-based imaging system (see Connor, these abstracts). Localized calcium concentrations were estimated from the ratio of images taken at 340 and 380 nm excitation. In resting cells growth cones and somatic blebs often showed higher calcium concentrations than the central soma. Stimulation via a microelectrode resulted in markedly higher calcium concentrations. These changes also showed regional localization. It will be of interest to determine whether manipulation of second messenger systems, including IP3, produces changes in the calcium signal or its localization in these neurons.

250.6 IONOPHORE A23187 AND VANADATE AS PROBES FOR $CA^{2+}$ STORES INVOLVED IN THE ACh-AND INOSITOL-1,4,5-TRISPHOSPHATE-EVOKED Cl-CURRENTS IN XENOPUS OOCYTES. B. Gillo*, I. Lotan*, R. Boton and Y. Lass. Department of Physiology and Pharmacology, Sackler School of Medicine, Tel-Aviv University Ramat-Aviv 69978, Israel.

The muscarinic responses in Xenopus oocytes consists of a transient and a long-lasting inward $Cl^-$ currents followed by an outward $K^+$ current. We have previously shown (Dascal,N., B.Gillo and Y.Lass, J. Physiol, in press, 1985) that the biphasic Cl current is mediated by an increase in the intracellular free Ca concentration, released from intracellular stores; intracellular Ca and inositol-1,4,5-trisphosphate (InsP$_3$;oron, Y., N.Dascal, E.Nadler and M.Lupu, Nature, 313:141-143,1985) injection mimic the muscarinic Cl current. In the present study we show that in Xenopus oocytes, pretreated with ionophore A23187, a dose dependent Cl current followed by an outward current are evoked upon raising external Ca concentration. The Cl current amplitude is reproducible during 40 min following the inophore application. Depletion of Ca stores by application of the ionophore in Ca-free solution, inhibits the acetylcholine reponse as well as the response to intracellular injecton of InsP$_3$; the transient Cl current being more sensitive to Ca depletion. Exposing the oocyte to orthovanadate in Ca free solution for 30 min. blocks both the acetycholine- and the InsP$_3$ - induced Cl currents. We suggest that Ca mediating the muscarinic Cl current in Xenopus oocytes is mobilized from intracellular store, probably the endoplasmic reticulum, by InsP$_3$.

250.7 **CALMODULIN-DEPENDENT PHOSPHATASE OF PC-12 PHEOCHROMOCYTOMA, C-6 GLIOMA, AND $GH_3$ PITUITARY ADENOMA CELL LINES.** L. Farber, F. Iannetta*, T. Kirby* and D. J. Wolff*. Department of Pharmacology, UMDNJ-Rutgers Medical School, Piscataway, NJ 088854.

Extracts of rat pheochromocytoma (PC-12), glioma (C-6), and pituitary adenoma ($GH_3$) cells depleted of endogenous calmodulin by DEAE-cellulose chromatography have been subjected to chromatography on immobilized calmodulin. Proteins eluted selectively at submicromolar $Ca^{2+}$ concentrations maintained with EGTA were found in each case to catalyze a calmodulin-stimulated dephosphorylation of myelin basic protein. Analysis of calmodulin-binding proteins by sucrose density centrifugation revealed that phosphatase activities exhibited sedimentation coefficients of 4.45, 4.23, and 4.20 for proteins derived from C-6, $GH_3$, and PC-12 cells respectively. Bovine brain calmodulin-dependent phosphatase, widely called calcineurin, exhibited a sedimentation coefficient of 4.63.

Polyclonal antibodies to bovine brain calcineurin in its native state, to the urea-dissociated A (62,000 $M_r$) subunit, and to the urea-dissociated B (16,000 $M_r$) subunit have been prepared in rabbits in our laboratory. These antibodies have been purified by chromatography on immobilized antigen. The affinity-purified antibodies have been examined for specificity by a Western blotting procedure. Antibodies derived from native calcineurin-injected animals showed reactivity to peptides of 62,000; 32,000; and 16,000 daltons. Antibodies derived from calcineurin A-injected animals showed reactivity to peptides of 62,000 and 32,000 daltons, while antibodies from calcineurin B-injected animals reacted with a single peptide of 16,000 daltons. Examination of calmodulin-binding proteins from PC-12, C-6, and $GH_3$ cells using antibodies directed against calcineurin B revealed cross reactivity with a peptide of 16,000 daltons.

These experiments support the contention that each of these cell lines contains a calmodulin regulated phosphatase homologous physically, kinetically, and immunologically to bovine brain calcineurin.

250.8 **REGIONAL DISTRIBUTION OF CALMODULIN-DEPENDENT PHOSPHATASE (CALCINEURIN) ACTIVITY IN RAT BRAIN.** E. Chung, M.Dvorozniak*, H.C. Li*, and M.H. Van Woert, Departments of Neurology, Biochemistry and Pharmacology, Mount Sinai School of Medicine, New York, NY 10029

Calcineurin, a major calmodulin binding protein found in the brain, possesses a calmodulin-dependent phosphatase activity towards phosphoproteins as well as nonprotein phosphosters such as p-nitrophenyl phosphate (PNPP). (Li,H.C. J. Biol. Chem. 259:8801, 1984). Calcineurin has been previously shown to be highly concentrated in the bovine striatum by radioimmunoassay. In the present study we have investigated the regional distribution of the PNP phosphatase activity of calcinineurin in the rat brain. Brain tissue was homogenized in 0.1 mM EGTA (pH 7.0) containing protease inhibitors, centrifuged 28,000 g for 20 min. and an aliquot of supernatant was incubated for 20 min at 30°C in 50 mM Tris HCl (pH7.4), 0.5 mM $NiCL_2$, 20 mM PNPP, and where applicable 0.1 mM $CaCl_2$, 0.3 uM calmodulin (CM) and 0.1 mM trifluoperazine (TFP).

It should be noted that TFP specifically abolishes the CM-activated phosphatase activity of calcineurin and $Ni^{2+}$ can serve as an activator for phosphatases in general.

PHOSPHATASE ACTIVITY
(nmoles PNP released/mg/min)

| | I EGTA | II $Ca^{2}$,CM,$Ni^{2+}$,TFP | III $Ca^{2+}$,CM,$Ni^{2+}$ |
|---|---|---|---|
| Striatum | 9.9±0.7 | 17.9±1.2 | 33.5±2.4 |
| Hippocampus | 7.6±0.5 | 15.1±0.6 | 25.1±1.3 |
| Cerebral Cortex | 11.6±0.6 | 17.1±0.6 | 27.5±1.4 |
| Midbrain | 10.7±0.6 | 9.4±0.2 | 12.5±0.4 |
| Hypothalamus | 13.7±0.7 | 16.3±1.2 | 17.4±0.9 |
| Cerebellum | 14.4±0.4 | 19.6±0.5 | 18.3±0.9 |
| Medulla | 11.0±1.0 | 14.3±1.4 | 14.8±0.9 |
| Spinal Cord | 9.3±0.6 | 11.7±0.7 | 10.5±0.6 |

Each value is the Mean + SE of 6 rats.

Basal phosphatase activity was measured in the presence of 1 mM EGTA and the values ranged from 7.6 (hippocampus) to 14.4 (cerebellum) nmoles PNP/mg/min. CM dependent phosphatase (calcineurin) activity (ColumnIII-Column II) was highest in the striatum. Hippocampus and cerebral cortex also exhibited high activity while the midbrain and hypothalamus had minimal activity.

The effect of destruction of nigro-striatal input by 6-hydroxydopamine and kainic acid lesion of the striatum on striatal calmodulin-dependent phosphatase activity will be described.

250.9 EXCITATORY POSTSYNAPTIC CURRENT (EPSC) OF THE SPINAL MOTONEURON. M. Kuno & S. Matsuura*. Dept. of Physiol., Osaka City Univ. Med. Sch., Osaka 545, Japan.

Dorsal root (DR) fibers and lateral column (LC) fibers have been suggested to contact on the different sites of the frog motoneuron on the basis of different time courses of the excitatory postsynaptic potentials (EPSPs). We applied voltage clamp technique to analyze the property of EPSCs induced by DR and LC stimulation and compared the response patterns between the EPSCs.

Frog spinal cord was isolated and intra-arterially perfused with oxygenated Ringer solution. The lumbar motoneuron was impaled by two separate microelectrodes filled with 4M K-acetate, of which tip distance was adjusted to 15-30 μm by using a microelectrode holder. After checking the impalement of two electrodes into the cell by injecting depolarizing current from either electrode and recording membrane potential (MP) by the other electrode, the MP was clamped at various levels between -130 and +60 mV. Monosynaptic and polysynaptic responses were evoked by LC and DR stimulation, respectively and the stimulus intensity was usually adjusted to produce almost the same size EPSPs.

In the successful recordings of the responses, the rise time of LC-EPSC estimated between 10-90 % of the amplitude was 2.8 ± 1.7 msec and 3.9 ± 2.5 msec for DR-EPSC. The mean time constant in the decay phase was 10.9 msec for LC-EPSC and 17.3 msec for DR-EPSC, when MP was clamped at the resting membrane potential level of the cell. The time courses were influenced by the level of holding potential: The EPSC rose and decayed faster at hyperpolarized holding potential than at the depolarized and resting MP levels. Reversal potential for the LC-EPSC was almost the same level as that for DR-EPSC in many cells. When spontaneous unit (miniature) EPSCs were recorded, the rise time and decay time were alittle faster than those of the EPSCs in response to LC and DR stimulation. The time courses also became much faster at hyperpolarized holding potential. Even in cells in which spontaneous miniature EPSPs were hardly discernible before and after the voltage clamp, clear discrete occurrences of unit EPSCs were observed when the MP was clamped at the level far from the reversal potential for the LC- or DR-EPSC. The unit EPSCs were often observed about 100-200 msec after stimulus presentation and after EPSCs evoked by LC or DR stimulation decayed to the base line noise level. The reversal potentials of the unit EPSCs were apparently the same as those of the EPSCs induced by LC or DR stimulation. Current-voltage relation also showed a similar pattern between the evoked EPSCs and the unit EPSCs.

We conclude from the study that LC-EPSC and DR-EPSC show essentially the same response property except for the faster time corse of LC-EPSC relative to DR-EPSC.

250.10 AMMONIA DECREASES THE SPINAL MONOSYNAPTIC REFLEX. W. Raabe. Depts. Neurology, VA Medical Center and Univ. of Minnesota, Minneapolis, MN 55417.

Ammonia intoxication allegedly plays a role in the pathophysiology of some metabolic encephalopathies. To investigate the neurophysiological effects of ammonia, the effects of ammonium acetate ($NH_4Ac$) i.v. on the spinal monosynaptic reflex were studied in decerebrated, paralyzed and respirated cats with the spinal cord severed at $L_2$.

$NH_4Ac$ i.v., 2-4 mmol/kg, decreased the spinal monosynaptic reflex elicited by stimulation of the hamstring, gastrocnemius or peroneal nerves and recorded from the ventral root $S_1$. $NH_4Ac$ had no effect on the excitability and conduction velocities of peripheral group I fibers and α-motoneuron axons, the group I fiber spike recorded from the dorsal roots at their entry into the spinal cord and the monosynaptic motoneuron population EPSP recorded from the ventral root. Intracellular recordings from spinal motoneurons confirmed that $NH_4Ac$ i.v. does not affect the monosynaptic EPSP.

These observations suggest that ammonia intoxication decreases the reflex discharge in the ventral root by decreasing the excitability of motoneurons and/or their intraspinal axons. Since neuronal activity increases the ammonia concentration in the CNS, ammonia may play a role as a metabolic feedback modifier which dampens or prevents excessive excitation of motoneurons (and possibly other neurons).

250.11 SOME EFFECTS OF NON-UNIFORM DISTRIBUTIONS OF SYNAPTIC CONDUCTANCE INPUTS ON SPINES AS MODELED IN A CORTICAL PYRAMIDAL CELL. W.R. Holmes* and C.D. Woody (SPON: R. Pay). Depts. of Biomathematics and Biobehavioral Sciences, UCLA, Los Angeles, CA. 90024.

Non-uniform distributions of two different types of synaptic conductance inputs have been shown theoretically capable of producing non-uniform resting potentials and changes in the efficacy of postsynaptic integration in layer V cortical pyramidal cells of cats (Holmes and Woody, Soc. Neurosci. Abstr., 1984). The efficacy of synaptic inputs depended on the driving force at the synaptic sites and the electrotonic distances from the soma to the synapses. The present study examines the effects of different distributions of activated conductances on the non-uniformity of resting potentials and the efficacy of synaptic inputs (as determined by peak transient soma potential) when dendritic spines are incorporated into the dendritic cable.

To do this a passive-membrane cable model of a neuron based on a serially reconstructed, HRP-injected cortical pyramidal cell was used. Symmetry assumptions were made to efficiently model the cell with 13,820 dendritic spines. Three types of inputs were modeled. Inhibitory inputs (I) with a reversal potential of -85mV were inserted on the soma and on proximal dendrites. Excitatory inputs (E) with a reversal potential of 0mV were distributed primarily on apical spines with some on basilar spines. M-current-like inputs (M) with a reversal potential of -85mV were placed primarily on basilar spines with some also on apical spines. The effects of different levels of synaptic activity within each region were evaluated while keeping soma resting potential and cell input resistance constant. These results were compared to those obtained with uniform distributions of E and M inputs on spines with and without I input on the soma and on proximal dendrites.

With I input on the soma and on proximal dendrites and uniform E and M input on spines, resting potential varied by up to 5mV within the neuron. When the E and M inputs were non-uniformly distributed, differences of 10-30mV could be seen between the resting potential at the soma and in distal dendrites. The efficacy of distal synapses as measured by peak transient or steady-state soma potential was 2.5 times greater with some input distributions than with others. It was also found that the effects of spines could be approximated in a model without spines by decreasing specific membrane resistance and increasing specific membrane capacitance in each segment according to the amount of membrane area that was added by spines.

(This research was supported in part by AFOSR and USPHS.)

250.12 AN ELECTROPHYSIOLOGICAL METHOD TO LOCATE VOLTAGE-SENSITIVE MEMBRANE ON DENDRITIC STRUCTURES. R.Malinow and J.P.Miller. Department of Zoology, University of California, Berkeley, CA 94720.

The integrative properties of a neuron may depend to a large extent on the existence and exact location of dendritic voltage-sensitive membrane. We are interested in determining if voltage-sensitive channels are located on spine heads, on the dendritic shaft or both. Furthermore, if active membrane resides on spine heads, we wish to know if the spine stem resistance provides enough electrical isolation to allow for locally regenerative voltage transients in the spine heads.

We have designed an experiment to determine the precise location of active dendritic membrane, and to evaluate its effect on synaptic integration. The experiments involve intra-dendritic recordings in combination with in situ laser photo-inactivation of dendritic regions. Computer simulations using compartmental models demonstrate that this experiment should, in fact, be capable of discerning between active membrane located on spine heads and on dendritic shafts. Furthermore, if spine heads do have active membrane, then the magnitude of the spine stem resistance may be deduced from the experimental results. Preliminary experiments have demonstrated the efficacy and reproducibility of using a laser to photo-inactivate and "transect" small portions of dye-filled neurons in the hippocampal slice preparation. Intracellular recordings during the microsurgical procedure confirm the transection and demonstrate the continued viability of the neuron.

250.13 A PHYSIOLOGICAL MECHANISM FOR A HEBB SYNAPSE J.P.Miller and R.Malinow. Dept. of Zoology, University of California, Berkeley, CA. 94720

Hebb postulated that the alteration of synaptic efficacy during classical conditioning depends upon the synchronous activity of the pre- and postsynaptic neurons. We propose a physiological mechanism by which such alterations might be achieved in spiny neurons. The necessary physiological "machinery" is : 1) the existence of regenerative, voltage-dependent channels on the heads of the dendritic spines of the postsynaptic neuron, and 2) some means through which a regenerative event localized to a single spine head could produce long lasting changes in synaptic chemistry and/or ultrastructure of that spine. One specific possibility would be as follows: 1) a voltage sensitive $Ca^{++}$ conductance might be present on the spine heads, and 2) a mechanism similar to that proposed by Lynch and Baudry (Science 224, 1057-1063, 1984) may operate to increase the density of active receptors on the spine head as a function of $[Ca^{++}]$. If the voltage threshold for a regenerative $Ca^{++}$ influx were relatively high, a significant (ie. $Ca^{++}$-buffer-saturating) increase in $[Ca^{++}]$ might result only when a synaptic input to the spine head was synchronous with a depolarizing voltage transient generated at some other location in the neuron. At the head of the spine, the EPSP from the direct input would sum with the voltage transient arriving from a spike or large EPSP generated at some other location in the cell, bringing the spine head membrane above threshold for regenerative $Ca^{++}$ influx. Following such a $[Ca^{++}]$ transient in the spine head, the efficacy of subsequent synaptic inputs onto the same spine would be increased, via an increase in active receptors. Repeated pairings of synaptic input with independent voltage transients would continue to increase synaptic efficacy. Though the hypothesis is speculative, compartmental models have been used to demonstrate the feasibility of this scheme. Details of these models will be presented, and the strengths and limitations of the hypothesis will be evaluated. W. Rall and I. Segev have performed similar simulations on clusters of active spines; we thank them for their valuable comments.

251.1 A MODEL FOR IN VIVO REGULATION OF PRODYNORPHIN AND PROENKEPHALIN. J. Eberwine, J. D. Barchas and C. J. Evans. Dept. of Psychiatry and Behavioral Sciences, Stanford University, Stanford, CA 94305.

The guinea pig adrenal gland is unusual in that processing products derived from both proenkephalin and prodynorphin are present in the same medulla cells. The neuroanatomical isolation of the adrenal gland makes this organ an excellent choice for the study of the expression of these two co-localized opioid precursors at both the protein and mRNA levels. In this study we have investigated the effects of hypophysectomy and dexamethasone administration on the expression of these two opioid precursors in male Hartley Albino guinea pig adrenal glands.

Slot blots and Northern blots were utilized to characterize and quantitate the changes in mRNA levels. Peptide levels were measured in acid extracts using radioimmunoassays directed to dynorphin(1-17), dynorphin B, and α-neoendorphin (prodynorphin products), met-enk-Arg-Gly-Leu, met-enk-Arg-Phe (proenkephalin products). Dexamethasone (0.1 mcg/kg administered via i.p. injection) increased in a time-dependent fashion the levels of proenkephalin mRNA 2-fold, however no detectable increase in prodynorphin mRNA level was observed suggesting that the mRNA of these two opioid precursors are differentially regulated. A slight increase in proenkephalin derived peptides was observed with little to no change in prodynorphin derived peptides. Hypophysectomy of the animals was found to have no discernable effect on either prodynorphin or proenkephalin mRNA or peptide levels.

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251.2 HISTAMINE IN THE RAT ADRENAL MEDULLA: IMMUNOCYTOCHEMICAL EVIDENCE FOR THE LOCATION OF HISTAMINE IN THENORADRENALINE-SECRETING ENDOCRINE CELLS. O. Häppölä*, S. Soinila, H. Päivärinta, T.H. Joh and P. Panula (SPON: O. Gandolfi). Dept. of Anatomy, University of Helsinki, SF 00170 Helsinki, Finland; and Lab Neurobiology, Cornell University Medical College, New York, New York 10021

Exogenously administered histamine results in a marked increase in both adrenal medullary and adrenal cortical secretion. There is pharmacological evidence that exogenous histamine exerts a direct action upon adrenal medullary cells and/or an indirect effect through splanchnic nerve stimulation, inducing catecholamine secretion, mainly adrenaline, from the adrenal medulla. Histamine-induced catecholamine secretion from the adrenal medulla is mediated primarily by the $H_1$ receptor. Histamine has been shown to depolarize the adrenal chromaffin cell membrane of several mammalian species. There is chemical evidence that histamine is present in the adrenal gland, while the location of histamine in the adrenal medulla is not known.

The present study was undertaken to examine the location of histamine in the rat adrenal medulla by an indirect immunofluorescence method using a specific histamine antiserum produced in rabbits. Twenty adult Sprague-Dawley rats were perfused through the left ventricle with 4% paraformaldehyde and an indirect immunofluorescence method was employed on 10 μM cryostat sections.

Histamine-immunoreactive cells, 10-20 μM in diameter, were observed in all rats studied. Most adrenal medullary cells were, however, non-reactive. No histamine-immunoreactive nerve fibers or adrenal cortical cells were detected. Mast cells were also stained, and they were easily distinguished from the adrenal medullary cells on the basis of size difference and morphology. Preabsorption with 50 μM histamine, but not with catecholamines or 5-hydroxytryptamine, completely abolished the staining. Studies of consecutive sections demonstrated that histamine-immunoreactive cell clusters showed no immunoreactivity to phenylethanolamine-N-methyltranserase, but they often showed intense immunoreactivity to dopamine-B-hydroxylase. Histamine-immunoreactive cell clusters were non-reactive to Met-Enkephalin$^5$-Arg$^6$-Phe$^7$ or 5-hydroxytryptamine.

It is concluded that the rat adrenal medulla contains non-mast cell histamine, which is located in the noradrenaline-secreting endocrine cells. It is possible that histamine is released from these cells and regulates the catecholamine secretion from the adrenal medulla.

251.3 BARIUM AND CALCIUM MEDIATE RELEASE OF CATECHOLAMINES FROM CULTURED CHROMAFFIN CELLS BY TWO DIFFERENT MECHANISMS. E. Heldman*[1], L. Raveh*[1], M.A. Levine*[2] and H.B. Pollard[2] (SPON: V. Teichberg). [1]Israel Institute for Biological Research, Ness-Ziona, Israel, and [2]Laboratory of Cell Biology and Genetics, NIADDK, NIH, Bethesda Md. 20205.

$BaCl_2$ evokes an intense secretory response when added to cultured chromaffin cells. It has been suggested that $Ba^{+2}$ may either serve as an analog to $Ca^{+2}$ or, alternatively, displace calcium from intracellular stores and thus produce the necessary intracellular environment for triggering the secretory process. We demonstrate here that $Ba^{+2}$ competes with calcium for the voltage-dependent calcium channels, but once it enters the cell, its mechanism of action is distinctly different from that of $Ca^{+2}$.

When increasing concentrations of $BaCl_2$ (0.1-3mM) were added to cultured chromaffin cells, increasing amounts of catecholamines were released depending on the $Ba^{+2}$ concentration. The kinetics of the secretion was long-lasting and resulted in depletion of up to 70% of the intracellular catecholamines. $CaCl_2$ significantly decreased the $Ba^{+2}$-induced catecholamine release. Complete inhibition of the $Ba^{+2}$-induced release was observed when the extracellular $Ca^{+2}$ to $Ba^{+2}$ ratio exceeded 2:1.

Kinetic studies with barium-133 revealed that the rate of $Ba^{+2}$ influx was also long-lasting (as for the $Ba^{+2}$-induced release) and was inhibited by extracellular $Ca^{+2}$. Nicotine (62μM) did not increase $Ba^{+2}$ influx but increased catecholamine release (in presence of 1mM $Ba^{+2}$), provided that at least 0.5mM $Ca^{+2}$ was present in the incubation medium. On the other hand, under the same conditions, 50mM KCl produced an increased $Ba^{+2}$ influx without any further increase in catecholamine release. Verapamil, high $Na^+$ (250mM), or $LaCl_3$ (2mM), compounds that block the voltage-dependent $Ca^{+2}$ influx, also blocked $Ba^{+2}$ influx as well as $Ba^{+2}$-induced catecholamine release. These data indicate that $Ba^{+2}$ enters the cell via the voltage-dependent channels and not via the nicotinic receptor-associated channels. The existance of these two types of calcium channels have previously been reported by us (E. Heldman et al, Soc. Neurosci., Vol 10, Abs 209.9, 1984).

While catecholamine release induced by nicotine, veratridine or high $K^+$ was greately inhibited by 10μM imipramine or 5μM TFP no effect of these drugs was observed on the barium-induced release. Unlike nicotine, veratridine or high $K^+$, which evoke co-secretion of catecholamines and ascorbic acid, barium did not evoke any release of ascorbic acid while producing intense secretion of catecholamines. In digitonin-treated cells, in the presence of 5mM EGTA, barium (0.1-1mM) also produced release which was further increased when $Ca^{+2}$ concentration was adjusted to 20μM.

Our data suggest that $Ba^{+2}$ and $Ca^{+2}$ compete for sites on the cell surface but within the cell they act differentially.

251.4 EXTRAADRENAL CHROMAFFIN TISSUE IN DOGS: DEVELOPMENTAL AND ULTRASTRUCTURAL CHARACTERISTICS. J.A. MASCORRO* (SPON: B. Hutchins). Department of Anatomy, Tulane University School of Medicine, New Orleans, LA 70112.

The catecholamine-containing chromaffin system consists of intra- and extraadrenal components. The extraadrenal portion may be paraaortic, paraganglion or intraganglion in position and represents a significant amount of the total system. The present work follows the development, anatomical distribution, structure and persistence of extraadrenal paraaortic chromaffin organs in dogs. Eight dogs aged 3 weeks (3), 6 weeks (2) or adult (3) were anesthesized and sacrificed by perfusion with glutaraldehyde. Their retroperitoneal tissue blocks (RTB) then were removed and immersed in a glutaraldehyde/potassium dichromate solution. This method produced a gross chromaffin reaction which clearly "mapped" the paraaortic organs and also rendered excellent preservation of the organs for morphological study. All animals demonstrated a voluminous system of "chromaffin tissue" in an extraadrenal paraaortic position. These chromated organs displayed a brown coloration, clearly visible to the unaided eye, typical of the chromaffin reaction and indicative of catecholamines. The paraaortic chromaffin bodies (PACB) were found in the midretroperitoneum and attained varying lengths. The youngest animals possessed PACB which varied from 21-35 mm in length. The 6 week dogs demonstrated PACB 20 mm and 31 mm long, respectively, while the PACB in adult animals attained lengths of 46 mm. Occasionally, single chromaffin bodies were evident elsewhere within the RTB, but the main paraaortic bodies represented the largest accumulation of extraadrenal chromaffin tissue. Histological observation showed that the paraaortic bodies were comprised of basophilic epithelial-type cells closely grouped and abundantly vascularized. Thin walled blood vessels together with connective tissue elements served to delineate the chromaffin cells into clusters. Electron microscopy corroborated these observations and confirmed the cytoplasmic presence of a multitude of dense catecholamine-like granules. Most granules exhibited homogeneously dense cores, however, certain vesicles appeared with cores which were lighter and granular. No evidence of innervation to the chromaffin cells was obtained even though much tissue was examined. It is apparent now that the extraadrenal chromaffin system in dogs is large, replete with a catecholamine-like product and persists through adulthood. It should be of paramount importance now to study this system and determine the response to stress factors which are known to cause catecholamine release from its intraadrenal chromaffin counterpart.

251.5 ULTRASTRUCTURAL DEMONSTRATION OF EXOCYTOSIS IN PERMEABILIZED ISOLATED BOVINE CHROMAFFIN CELLS. S.W. Carmichael and J.C. Brooks (SPON: J.R. Daube) Dept. of Anatomy, Mayo Clinic, Rochester, MN 55905 and Dept. Basic Sciences, Marquette University School of Dentistry, Milwaukee, WI 53233.

Exocytosis is the direct discharge of the contents of an intracellular vesicle into the extracellular space. Although there is overwhelming biochemical evidence implicating exocytosis as the primary mechanism for the secretion of catecholamines, proteins, and nucleotides from chromaffin cells, there has been a paucity of ultrastructural evidence of exocytosis. Except for studies of the hamster, transmission electron microscopic techniques have not been able to unequivocally resolve the event. We examined cultured bovine chromaffin cells using a modification of the method of Buma, et al. (Histochem. 80:247, 1984) which employs tannic acid in the culture medium. As the contents of the chromaffin vesicle are exposed to the medium, they are apparently coagulated in situ. In parallel biochemical studies it was noted that catecholamines were not measureable in the culture medium in the presence of tannic acid but could be measured when tannic acid was not present. The exposed contents appear more granular than vesicles in the interior of the cell. The process of exocytosis appears to be stopped as the contents are exposed to the tannic acid, and many images of exocytotic profiles have been captured. The plasma membrane of cells treated with tannic acid is also very distinct. Some cells were treated with the detergent saponin, which has been shown by SEM to produce holes in the plasma membrane (Brooks and Carmichael, Mikroskopie 40:347, 1983). We have demonstrated these holes by TEM. Furthermore, in saponin-treated cells that were stimulated with 100μM calcium, exocytosis was seen to occur at the intact plasma membrane and not at the holes.

This work was supported by the Mayo Foundation and NSF Grant No. 8NS-8304154.

251.6 DRUG MODIFICATION OF CATECHOLAMINE SECRETION AND THIOPHOSPHORYLATION IN PERMEABILIZED CHROMAFFIN CELLS. J.C. Brooks and M. Brooks*. Department of Basic Sciences, Marquette Univ. Sch. of Dent., Milwaukee, WI 53233.

Chromaffin cells permeabilized with saponin secrete by an exocytotic mechanism that requires exogenous calcium and has partial dependence on exogenous ATP. Replacement of ATP by the ATP analog adenosine-5'-O-(3-thiotriphosphate) (ATPγS) irreversibly thiophosphorylates cell proteins and permanently inhibits secretion. This secretory inhibition is associated primarily with the thiophosphorylation of 43 and 54 kilodalton (kda) intracellular proteins. The 43 kda protein shows a greater incorporation of $^{35}S$ from [$\gamma$-$^{35}S$]ATP in the presence of calcium. We have examined the effect of drugs which influence cellular phosphorylating systems and specific organelles on the pattern of thiophosphorylation and catecholamine secretion by permeabilized cells.

Several kinase activators and inhibitors were examined for their influence on thiophosphorylation of the 43 kda protein and catecholamine secretion. Trifluoperazine, cAMP and cGMP had no effect on stimulated catecholamine secretion. Trifluoperazine reduced total $^{35}S$ incorporation into cells but had no effect on incorporation into the 43 kda protein. Incorporation of $^{35}S$ into this protein was slightly reduced by cAMP and its inhibitor protein in the presence of calcium. Phorbol 12-myristate 13-acetate slightly enhanced catecholamine secretion with a reduction of $^{35}S$ incorporation into the 43 kda protein.

Inhibitors of cytoskeletal filaments and microtubules, cytochalasin B and colchicine, had no effect on catecholamine secretion. Similarly, several drugs known to influence chromaffin granule function had no effect on secretion. This included dicyclohexylcarbodiimide inhibition of the proton-translocating ATPase, rotenone inhibition of catecholamine uptake and atractyloside inhibition of nucleotide uptake into granules. Atractyloside, however, prevented $^{35}S$ incorporation into the 43 kda protein, suggesting a role for this protein in granule function.

This work was supported by NSF Grant No. BNS-8304154.

251.7 REGULATION OF TYROSINE HYDROXYLASE ACTIVITY IN THE ADRENAL MEDULLA OF THE OVINE FETUS. C. Y. Cheung. Dept. of Reproductive Medicine, Univ. of Calif., San Diego School of Medicine, La Jolla, CA. 92093

In the ovine fetus, the adrenal medulla (AM) is functionally active, and secretes catecholamines at a high rate as term approaches. We have found that the ovine fetal AM contained and secreted more catecholamines than that of the adult. The present study was designed to investigate the regulation of the catecholamine biosynthetic enzyme tyrosine hydroxylase (TH) in the AM of the fetus. Ovine fetuses at 130-135 days gestation were used. The ewe was anesthetized, the fetus exteriorized, and the adrenal gland rapidly removed. The medullary tissue was homogenized in MES buffered saline and centrifuged at 49,000Xg for 1 hour. TH in the supernatant was measured using the method of Waymire et al. (Anal Biochem, 43:588, 1971). The kinetic constants of fetal TH for tyrosine, determined in the presence of excess cofactor, were Vmax= 2.9±1.0 pmol $CO_2$/min/ug protein and Km=278±130 uM. These values were significantly higher than the adult values of 1.5±0.3 pmol/min/ug and 46±3 uM. Similarly the kinetic constants of the fetal enzyme for cofactor were Vmax=5.1±1.5 pmol/min/ug and Km=174±42 uM, significantly higher than the 0.2±0.04 pmol/min/ug and 37±11 uM measured in the adult. Furthermore, the activity of fetal TH was not inducible by 100uM acetylcholine even in the presence of $CaCl_2$. These results contrasted with those seen in the adult where 100uM acetycholine stimulated TH activity by 59% in the presence of 1.5mM $CaCl_2$ ($p<0.05$). We have previously shown that prolactin and VIP could increase catecholamine release from fetal adenomedullary cells in culture. To determine whether these hormones could regulate TH activity, the fetal enzyme was incubated with 40 ug/ml ovine prolactin or $10^{-4}$M VIP for 30 minutes, and assayed for activity. Prolactin and VIP significantly increased the activity of fetal TH by 24% and 51%, resp. These results suggest that in the AM of the ovine fetus, the greater catecholamine content and release rate was associated with higher activity of the biosynthetic enzyme TH, compared to the adult. In addition, the fetal enzyme was not responsive to cholinergic stimulation while inducible by prolactin and VIP, suggesting that catecholamine synthesis in the fetal AM may be more responsive to endocrine regulation than to sympathetic neural stimulation.

251.8 GLUCOCORTICOIDS REGULATE DOPAMINE SYNTHESIS IN BOVINE ADRENAL CHROMAFFIN CELLS BY INCREASING STIMULUS-INDUCED PHOSPHORYLATION OF TYROSINE HYDROXYLASE K.L. Kelner, K.W. Brocklehurst*, H.B. Pollard and D.M. Kuhn+ Lab of Cell Biology and Genetics, NIADDK, and +Section on Biochemical Pharmacology, NHLBI, NIH, Bethesda, MD 20205.

We have previously shown that maintenance of isolated bovine adrenal chromaffin cells with glucocorticoids increases cellular dopamine levels. This is due to a glucocorticoid-induced increase in the *in situ* activity of tyrosine hydroxylase (TH). When the cells are stimulated with secretagogues, dopamine synthesis and TH activity increase in control cells, but these increases are much larger in cells that have been pretreated with glucocorticoids (Kelner, Levine, Morita and Pollard, 1985). Since secretagogues are known to increase TH activity by stimulating phosphorylation of the enzyme, which increases the affinity of TH for its cofactor, tetrahydrobiopterin, we tested whether glucocorticoid pretreatment of chromaffin cells increased stimulation-induced TH phosphorylation.

Chromaffin cells treated for 3 days with 10 μM dexamethasone were prelabeled for 90 min with $^{32}Pi$ and then exposed to various secretagogues for 2 min. Total cellular proteins were separated by SDS-PAGE and the phosphoproteins detected by autoradiography. In a parallel set of samples, TH was immunoprecipitated from cell lysates and the amount of $^{32}P$ incorporated into TH was quantitated either by SDS-PAGE, autoradiography and densitometry or by direct scintillation spectroscopy on the immunoprecipitated pellets. Glucocorticoids caused a small increase in the basal phosphorylation of TH which paralleled the increase in basal TH activity. The secretagogues, veratrine (50 μM), KCl (65 mM) and carbachol (300 μM) increased phosphorylation of TH and, as shown previously, its *in situ* activity. However, in cells pretreated with glucocorticoids, the stimulation-induced phosphorylation was markedly augmented, as was *in situ* TH activity. The correlation between glucocorticoid-induced increases in TH phosphorylation and *in situ* TH activity suggests that glucocorticoids regulate TH activity by increasing stimulation-induced phosphorylation of the enzyme.

251.9 SUPPLEMENTATION OF ADRENAL CHROMAFFIN CELLS WITH COFACTORS REQUIRED BY CATECHOLAMINE (CA) BIOSYNTHETIC ENZYMES CAN INFLUENCE CA BIOSYNTHESIS.
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Primary cultures of adrenal medullary chromaffin cells have been used to study the regulation of the enzymes involved in CA biosynthesis by their respective cofactors. The following cofactors were added to cultured chromaffin cells: 1) tetrahydrobiopterin ($BH_4$), the cofactor for tyrosine hydroxylase (TH); 2) ascorbic acid (AA), the cofactor for dopamine-B-hydroxylase (DBH), and 3) s-adenosylmethionine (SAM), the cofactor for phenylethanolamine-N-methyl transferase (PNMT). CA biosynthetic rates were determined by monitoring the incorporation of $^{14}$C-tyrosine (tyr) into intracellular CA by reverse-phase HPLC.

Incubation of cells with various concentrations of $BH_4$ for up to 2 hours in the presence of 20 uM $^{14}$C-tyr stimulated $^{14}$C-dopamine (DA) formation, which saturated at approximately 500 uM $BH_4$. However, $BH_4$ almost totally blocked the incorporation of $^{14}$C into cellular NE at this saturating concentration. AA (2 mM) alone was able to stimulate the rate of $^{14}$C incorporation into NE from $^{14}$C-tyr within the same time frame. Incubation of cells in the presence of SAM at 200uM significantly increased the rate of $^{14}$C accumulation into epinephrine. This SAM-dependent increases was seen only after much longer times (up to 6 hours)

The inhibitory effect of $BH_4$ on NE biosynthesis at higher concentrations was investigated further by incubating the cells with a combination of $BH_4$ and AA. AA (2 mM) was not able to reverse the inhibition of $BH_4$ on NE biosynthesis in the chromaffin cell model. It is possible that $BH_4$ is able to compete with AA at some site involved in the transfer of electrons to DBH within the granule. Thus, $BH_4$ may serve as a tool to probe the mechanism by which AA is coupled to DBH in NE biosynthesis. Additionally, since $BH_4$ is currently being tested in clinical trials for the treatment of certain neurological and psychiatric illnesses, it will be important to determine if during $BH_4$ therapy there is any inhibition of NE biosynthesis either in the periphery or in the central nervous system.

251.10 HISTOCHEMICAL AND BIOCHEMICAL STUDIES OF GABAERGIC SYSTEM IN THE ADRENAL MEDULLA. M. Fujimoto*, I. Hanbauer, H. Alho* and A. Guidotti. Lab.Preclin.Pharmacol,NIMH, St. Elizabeths Hosp.,Washington, D.C. and Hypertension Endocrine Br., NHLB Inst., NIH, Bethesda, MD 20205 (SPON: L.R. Steranka)

Cultured chromaffin cells of bovine adrenal medulla contain alpha-aminobutyric acid (GABA), glutamic acid decarboxylase (GAD), GABA aminotransferase, and GABA receptors. Kataoka et al. showed that nicotinic receptor stimulation releases not only catecholamines (CA), but GABA from the chromaffin cells and that GABA modulates the release of CAs (PNAS 81: 3218, 1984). Further detailed histochemical studies of adrenal glands of several mammalian species indicated that GAD immunoreactivity is present not only in the chromaffin cells but predominantly in nerve fibers. In bovine adrenal the GAD immunoreactivity is mainly located in bundles of nerve fibers, innervating the medulla. In dog adrenal a dense network of GAD positive fibers is localized in the boundary between cortex and medulla. In rat medulla, GAD immunoreactive fibers surround chromaffin cells and blood vessels. To establish the origin of fibers showing GAD positive immunoreactivity, we examined the activity of GAD in rat adrenal medulla 10 days after splanchnic nerve transsection. The enzyme activity in the denervated medulla was reduced by 10%, and GAD immunoreactive fibers were still present indicating that a large part of GAD containing fibers arriving at the medulla are not associated with the splanchnic nerve. In vivo studies with male Foxhound dogs were performed to evaluate the functional role of GABA in adrenal medulla. Drugs were injected directly to the gland by the technique described by Hilton et al. (Am.J. Physiol. 192: 525, 1958) and blood samples to measure CAs and Met$^5$-enkephalin-like immunoreactivity (ME-LIR) were obtained from the left adrenal vein. THIP ($GABA_A$ receptor agonist) increased the release of CAs and ME-LIR in parallel. Chromatographic profiles of the plasma samples on Sephadex G75 indicated that ME-LIR was associated with larger molecular forms of ME. These profiles were very similar to those of samples obtained after splanchnic nerve stimulation. Disappearance of ME might be due to the rapid degradation during the preparation of plasma samples. The release of CAs and ME-LIR was not blocked by hexamethonium, naloxone or splanchnicotomy, but was instead prevented by bicuculline. These data suggest that GABA-induced release of CA and ME-LIR were the consequence of chromaffin cell depolarization following direct stimulation of $GABA_A$ receptors located on chromaffin cell membranes. In fact, THIP ($10^{-3}$M) produced a significant release of CAs from cultured chromaffin cells providing that the $Cl^-$ concentration in the incubation medium is maintained under 50 mM.

251.11 DISTRIBUTION OF MONOAMINE OXIDASE TYPES A AND B IN THE ADRENAL GLAND. G.L. Pfeiffer* and S.W. Carmichael. Dept. of Anatomy, Mayo Clinic, Rochester, MN 55905.

Biochemical studies have shown that the chromaffin cells of the bovine adrenal medulla contain only the B type of monoamine oxidase (MAO-B) (Youdim, M.B.H., et al., Soc. Neurosci. Abstr., Vol 10, Part 2, p. 722, 1984). Furthermore, Youdim et al. showed that cells from a tumor of the rat adrenal medulla (PC12 cells) contained only the A type of the enzyme (MAO-A). In a histochemical study we have localized the enzyme in the adrenal gland of cow, man, dog, cat, and rat. For the histochemical method, tetranitroblue was used as the stain and tryptamine was the substrate. Specific inhibitors for MAO-A (clorgyline, $10^{-7}$M) and MAO-B (deprenyl, $10^{-7}$M) were used prior to and during the staining reaction. Pargyline ($10^{-2}$M), heat and substrate-free medium were used as controls, and these showed the method to be valid. Our findings confirmed that the bovine chromaffin cells contain MAO-B and endothelial cells contain MAO-A. The distribution of MAO-B activity is heterogeneous within the medulla. More activity could be seen surrounding the central vein than in peripheral parts of the medulla. Nerves within the bovine adrenal medulla and cells surrounding these nerves contain both types of MAO. The adrenal medullas of man, dog, cat, and rat were not as reactive as the cow--indicating less enzyme activity in these species. Preliminary results on human pheochromocytoma (an adrenal medulla tumor) indicate that MAO-A is predominant. Within the adrenal cortex there is a distinct zonation for the two types of MAO that varies among species. The zona glomerulosa of the cow adrenal cortex contains MAO-B whereas the zona fasciculata and zona reticularis contain MAO-A. The adrenal cortex of the other species studied contain mostly MAO-B, except the canine zona reticularis which also contains MAO-A.

251.12 DIHYDROPTERIDINE REDUCTASE IS PHOSPHORYLATED BY CYCLIC-AMP DEPENDENT PROTEIN KINASE FROM BOVINE ADRENAL MEDULLA. Keith W. Brocklehurst*, Robert A. Levine+, Kyoji Morita++, Sheldon Milstien++, and Harvey B. Pollard. Lab of Cell Biology and Genetics,NIADDK, and ++Lab of Neurochemistry, NIMH, NIH, Bethesda, MD 20205
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Dihydropteridine reductase (DHPR) plays an integral role in biogenic amine synthesis by converting quinoid-dihydrobiopterin (q-$BH_2$) to tetrahydrobiopterin ($BH_4$). $BH_4$ is the electron donor for phenylalanine, tyrosine (TH), and tryptophan (TrpH) hydroxylases in the process of aromatic amino acid hydroxylation in various tissues. It has been thought that DHPR is normally in excess in most tissues since the majority of biopterin is present in the fully reduced, tetrahydro form ($BH_4$). Since phosphorylation appears to be a critical factor for the short-term activation of both TH and TrpH (by different mechanisms), we have investigated whether purified sheep liver DHPR could be phosphorylated by any of the known protein kinases found in adrenal medulla. A rabbit antibody to DHPR was used to examine specific incorporation of $^{32}$P into DHPR.

An adrenal medullary post-microsomal supernatant was prepared in the presence of 1 mM EGTA to be used as a source of kinases. DHPR was incubated (3 min, 37°C) in the presence of medullary supernatant, [gamma-$^{32}$P]ATP, and the following activators of protein kinases: 1) c-AMP; 2) c-GMP; 3) $Ca^{+2}$; 4) $Ca^{+2}$, calmodulin, and 5)$Ca^{+2}$, phosphatidylserine. After the reaction, aliquots were subjected to SDS-PAGE and autoradiography. Marked phosphorylation of a protein of approximately 28,000 $M_r$ corresponding to exogenous DHPR occurred only in the presence of c-AMP. Immunoprecipitation with the antibody and protein A confirmed that $^{32}$P was incorporated into DHPR. Thus, under these in vitro conditions, DHPR can be phosphorylated by a c-AMP dependent kinase, but not by protein kinases that are dependent on other activators. These studies reveal the possibility that c-AMP-dependent phosphorylation of DHPR could play a role in regulating the initial hydroxylation reactions in biogenic amine synthesis.

252.1 **EFFECTS OF UNILATERAL BRAIN DAMAGE ON THE IMMUNE SYSTEM IN MICE AND MAN.** D.M. Nance, R. Carr* and P.W. Nance*. Departments of Anatomy, Rheumatology, Physical Medicine and Rehabilitation, Dalhousie University, Halifax, Nova Scotia B3H4H7, Canada.

Based upon the effects of unilateral hypothalamic lesions we have proposed the presence of a functional brain asymmetry in the neuroendocrine control of the gonads (B.R.B. 13:651,84). Renoux et al (J. Neuroimmunol. 5:227,83) have reported that the effects of unilateral cortical ablation on T-cell function in mice depends upon which hemisphere is lesioned. We have examined the effects of left or right unilateral cortical aspiration in mice on the in vitro lymphoproliferative response of spleen cells to mitogens. Additionally, specific antibody titers in mice with unilateral cortical lesions were measured following immunization with ovalbumin. Finally, in human subjects with predominately left or right cerebral damage the in vitro response of peripheral lymphyocytes to mitogens was tested. Histologically the cortical aspirations in the mice were on average localized to a dorsomedial strip of cortex 1.2 mm wide and extended from the frontal cortex posteriorly 5.5 mm. In female mice, unilateral cortical lesions had no detectable effect on the mitogenic response of spleen lymphocytes to concanavalin A and phytohemagglutinin. Similar assays in humans indicated a tendency ($p<.072$) towards increased T-cell function in subjects with left cerebral damage. Finally, antibody titers following immunization in mice indicated a significant sex difference in titers (female>male, $p<.001$) and a tendency ($p<.078$) in both male and female mice with right cortical lesions towards reduced antibody production. The female mice with right cortex lesions had larger thymus glands than sham and right cortex lesion groups ($p<.014$). Male mice with right cortex lesions were the only group not to gain body weight during the study ($p<.024$). There was a sex difference in adrenal weight (female>male, $p<.001$) and in the female lesion groups the adrenal gland was smaller ($p<.05$) on the same side as the brain lesion relative to the contralateral side of the body. In general, these results support the possibility of a functional asymmetry in the neural control of the immune system. The direction of the changes in immune function reported here are opposite to those reported by Renoux (1983). The smaller size and dorsomedial location of the present lesions may account for these differences and suggests there may be further segregation of function between various viscero-cortical regions. Functional asymmetries in the neural control of both the endocrine and immune systems may further reflect an underlying asymmetry in the organization of the autonomic nervous system. Supported by the Medical Research Council of Canada and the Dalhousie Medical Research Foundation.

252.2 STEREOSPECIFICITY OF OPIATE IMMUNOSUPPRESSION IN RATS. F.C. Martin, Y. Shavit, G.W. Terman, R.N. Pechnick, C. Oh*, and J.C. Liebeskind. Neuroscience Program and Department of Psychology, UCLA, Los Angeles, CA 90024.

There is growing experimental evidence that both endogenous and exogenous opioids affect immune function. Our laboratory has been studying the effects of opioids on natural killer (NK) cells, lymphocytes that spontaneously recognize and kill tumor cells and are present in blood, lymph nodes, and spleen. We have demonstrated that both systemic morphine and endogenous opioids released by stress suppress NK function in rats (Y. Shavit et al., Science 223:188, 1984). More recently, we found that the suppression caused by 30 mg/kg of systemic morphine can be reproduced by giving only 20 µg intracerebroventricularly, suggesting that morphine's effect is centrally mediated.

The present study examined the stereospecificity of opiate-induced NK suppression by comparing the opiate drug levorphanol (LEV) with its inactive stereoisomer dextrorphan (DEX) in vitro and in vivo. Subjects were 12-18 week old male Fischer 344 rats. For in vivo studies, rats were given DEX or LEV (10 mg/kg, s.c.) and splenectomized 3 hrs later under anesthesia. Spleens were homogenized into single cell suspensions and these cells mixed with chromium-51 labeled YAC-1 tumor target cells. NK activity was then measured in a 4h chromium release assay. For in vitro studies, spleen cells were incubated in cell culture media containing LEV or DEX for 3 hrs at 37°C before being washed and assayed as above.

SPECIFIC NK CYTOTOXICITY (% OF SALINE CONTROL) MEAN ± 95% CI

| Dose | Dextrorphan | Levorphanol | |
|---|---|---|---|
| in vivo | | | |
| 10 mg/kg | 98±13 | 61±10 | $p < 0.001$ |
| in vitro | | | |
| 5x10-4M | 4+5 | 11+14 | NS |
| 5x10-5M | 61±28 | 58±22 | NS |
| 1x10-6M | 92±21 | 88±22 | NS |

These results indicate that LEV but not DEX suppresses NK activity in vivo. However, in vitro these drugs produced a non-stereospecific suppression apparent only at high concentrations, suggesting this effect was not mediated by binding to opiate receptors.

We conclude that the opiate alkaloids, morphine and LEV, do not suppress NK activity in vivo by acting directly upon NK cells. Rather, these results support our previous findings indicating that this effect depends upon binding to central opiate receptors. (Supported by NIH grant NS-07628).

252.3 THE STIMULATORY EFFECTS OF NERVE GROWTH FACTOR ON IN VITRO LYMPHOCYTE PROLIFERATION. L.W. Thorpe*, C. Beck* and J.R. Perez-Polo, Dept. Human Biol. Chem. and Genetics, Univ. Tex. Med. Br., Galveston, TX., 77550.

It has been shown that numerous hormones and neurophysiological agents play an important role in immunoregulation. Specific receptors for many of these substances have been demonstrated on lymphophocytes and the immunomodulatory effects of these neurohormonal agents is initiated through receptor ligand interaction. Nerve growth factor (NGF) is a neuronotrophic peptide important in the development, maintenance and regeneration of sympathetic and sensory neurons. We have recently demonstrated the existance of receptors on the surface of both human and rat lymphocytes. These findings suggest that NGF may also participate in the regulation of immune function. To test this hypothesis we examined the lymphoproliferative effects of mouse β-NGF on rat splenic cells in culture. Varying concentrations of NGF (1-20 ul/ml) were added to triplicate microtiter plate cultures and incubated for 48-120 hours. In addition, NGF (1-10 ug/ml) was also added to cultures containing Con A (1 ug/ml) and cultured for 96-120 hours. Proliferative activity was measured by [$^3$H] thymidine (Thd) incorporation. NGF had no effects on [$^3$H]Thd uptake at either 48 or 72 hours. By 96 hours of culture, NGF resulted in a significant stimulation of lymphoproliferative activity ($p<0.001$). Optimal stimulation by all concentrations of NGF was seen at 120 hours ($p<0.01$) The data are shown here in tabular form as the mean CPM±standard deviation.

| Hrs. in Culture | [NGF] ug/ml 0 | 1 | 5 | 10 | 20 |
|---|---|---|---|---|---|
| 96 | 170±28 | - | 678±88 | 935±102 | - |
| 120 | 218±60 | 435±47 | 1568±324 | 1512±211 | 1307±37 |

NGF also showed a dose dependant augmentation of Con A induced [$^3$H]Thd uptake at 120 hours.

| | CPM±SD | |
|---|---|---|
| Con A (1ug/ml) | 21656±1802 | |
| Con A+1ug/ml NGF | 31040±5692 | $p<0.05$ |
| Con A+10ug/ml NGF | 54507±5126 | $p<0.01$ |

These results suggest that, at least **in vitro**, NGF enhances the lymphoproliferative response of rat spleen cells and also is synergistic with other mitogenic stimuli. The role that NGF may play in the **in vivo** immune response remains to be elucidated. Supported by NIH Grant NS18708 and Robert Welch Grant H698.

252.4 NEURAL REGULATION OF CELLULAR IMMUNE FUNCTION IN MICE. C.A. Mullen*, J.D. Hirsch, and B. Beer. Dept. of CNS Res., Medical Research Division of American Cyanamid Co., Lederle Labs, Pearl River, NY 10965.

The central nervous system is now known to communicate anatomically and chemically with the immune system. We investigated this interaction by determining the effects of autonomic neurotransmitters on selected aspects of the murine cellular immune response. Initial experiments demonstrated that parasympathetic stimulation (PS) in vivo induced by physostigmine (250 µg/kg s.c.) caused an increase in vitro in both mitogen-provoked interleukin-1 (IL-1) and interleukin-2 (IL-2) production while sympathetic stimulation (SS) induced by norepinephrine (750 µg/kg i.p.) exerted the opposite effect. Both physostigmine and norepinephrine were administered twenty minutes prior to removal of the spleen or thymus. In addition, thymocytes and splenic lymphoblasts from parasympathetically-stimulated mice were less responsive to murine and human IL-1 and murine and rat IL-2, respectively. Based upon these results, we expanded our studies to investigate more appropriate in vitro models of cellular immunoreactivity: 1) the allogeneic mixed lymphocyte reaction (AMLR); 2) allogeneic cell-mediated cytotoxicity (CTX); and 3) natural killer cell (NK) activity. In the AMLR, the proliferation of C57BL/6 responder splenocytes to alloantigens on γ-irradiated BALB/c stimulator cells was enhanced by prior PS but reduced subsequent to SS. In contrast, both PS and SS enhanced allogeneic CTX using $^{14}$C-labeled BALB/c Con A-lymphoblasts as target cells. In addition, the enhancement by PS of allogeneic CTX was partially blocked by atropine, a muscarinic cholinergic antagonist, given to C57BL/6 mice at 10 mg/kg i.p. thirty minutes prior to the administration of physostigmine. Neither PS nor SS had any acute effects on NK activity in freshly isolated spleen cells from C57BL/6 or CBA/CA mice. NK activity was measured by a $^{51}$Cr-release assay with YAC-1 mouse lymphoma cells as targets. However, prior PS in CBA/CA mice followed by overnight culture of unfractionated spleen cells resulted in an increase in residual NK activity compared to controls which was observed in the presence or absence of the interferon-inducer, polyinosinic:cytidilic acid, a known in vitro stimulator of NK activity. These results suggest that the autonomic nervous system (ANS) may play a key role in modulating interleukin production and target cell response to these immunoregulatory proteins. The ANS may thus influence the activity of various cells involved in the cellular immune response through this mechanism. These results also imply that it is possible to exploit the potent immunoregulatory effects of the nervous system in designing drugs for a variety of therapeutic areas.

252.5 **FEVER AND NEUTROPHILIA INDUCED IN RATS FOLLOWING THE CENTRAL ADMINISTRATION OF HIGHLY PURIFIED HUMAN INTERLEUKIN-1.**
G.J. Patel*, P.C. Harrison*, V.B. Ciofalo and R.B. Faanes*. Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT 06877.

**The monokine Interleukin-1 (IL-1) is one of the products of mononuclear phagocytes. It is synthesized and released from these cells in response to stimuli such as microorganisms and microbial products. IL-1 is capable of gaining access from the periphery to the thermoregulatory centers of the hypothalamus in the brain to initiate fever. Peripheral administration of IL-1/endogenous pyrogen/leukocytic endogenous mediator has been reported to produce fever and accelerate release of mature neutrophils (neutrophilia) from the bone marrow.**

**In this study, we report that direct administration of highly purified human IL-1 into the rat brain results in both fever and neutrophilia. IL-1 (1 unit), heat-inactivated IL-1 or saline (10 µl each) was administered into the third ventricle of conscious adult rats via a stereotaxically implanted guide cannula. Core temperature was measured at 30, 60, 120 and 180 min. post-treatments. Peripheral blood was collected from retro-orbital sinus 180 min. after IL-1 or saline administration for a neutrophil count.**

**IL-1 produced a highly significant increase in body temperature of rats (N=14) within 30 minutes of administration as compared to saline controls (N=15). The peak increase (mean 1.2 ± 0.07°C) was recorded at 30 min. Significant febrile response was also observed at 60, 120 and 180 min. (mean 1.1 ± 0.07; 0.82 ± 0.11; 0.75 ± 0.14°C, respectively) in IL-1 treated rats. Heat inactivated IL-1 failed to initiate fever in rats (N=5).**

**IL-1 also produced a significant increase in the number of circulating peripheral blood neutrophils (mean 3034 ± 423, N=6) as compared to saline controls (1534 ± 217, N=6). In a separate experiment, peripheral blood neutrophils were counted following the administration of half the amount of IL-1 (1/2 unit) or saline (5 µl each) into the lateral ventricle of the rat brain. In this experiment also, IL-1 produced a similar increase in the number of circulating neutrophils (mean, IL-1, 4110 ± 591 vs. saline, 1497 ± 261, N=4 each). These results suggest that purified human IL-1 is capable of producing host-defense responses such as fever and neutrophilia through a CNS mechanism.**

252.6 IMMUNOLOGICAL REACTIVITY FOLLOWING ACUTE AND CHRONIC STRESS.
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Considerable evidence suggests that psychosocial factors such as stressors can modulate immune function and resistance to disease. Stress-induced changes in the CNS resulting in altered autonomic and neuro-endocrine outflow are most likely responsible. CNS responses to stressors are influenced by both the nature of the stimulus (form, severity, chronicity, controllability) and the organism (genetics, age). It is therefore of interest to determine how these factors influence the response of the immune system to stress.

Here, we examined the effects of acute vs. chronic footshock stress on several measures of immune function in 3 strains of mice. Male mice were exposed to 1 of 3 stress conditions which we previously found to induce consistent alterations in central noradrenergic pathways: (1) 14 daily 1.1 hr sessions of intermittent inescapable footshock (Chronic stress); (2) One session of footshock (Acute stress); or (3) Exposure to the apparatus (No shock controls). One day later, mice were sacrificed and their spleens taken for measurement of lymphocyte reactivity in vitro. We examined the cytotoxic activity of natural killer (NK) cells, the proliferative responses of T and B cells, and the generation of cytotoxic T lymphocytes following stimulation.

Acute shock reduced the level of NK cell activity in CD-1 and C57BL/6 mice. In contrast, chronically stressed mice of these strains exhibited enhanced NK function. This augmented activity was depressed by fighting behavior. No significant changés in NK function were observed in DBA/2 mice. In CD-1 mice, lymphocyte proliferative and T cell cytotoxic functions were not affected by the stress regimens. Together the results of the present experiments suggest that, in addition to the well-known immunosuppressive effects of certain forms of acute stress, compensatory changes occurring under conditions of chronic stress may lead to augmentation in activity of certain cells of the immune system, depending on the strain of mice. These findings have important implications for understanding the complex relationships between stress and infectious and malignant disease.

252.7 CEREBRAL LATERALIZATION: ASSOCIATION WITH SERUM ANTIBODIES TO DNA IN SELECTIVELY BRED MOUSE LINES.
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Lateralization of function, particularly handedness, has long been associated with cerebral asymmetry and an increased incidence of CNS-related disorders. Geschwind & Behan (1982) have reported an increased frequency of migraine, developmental learning disabilities, and immune-related disorders among strongly left-handed subjects. The latter finding is unexpected for associations of handedness with non-CNS related phenomena are rare. Geschwind & Behan explained this association by postulating that androgen exposure, controlled by MHC genes, influenced both the development of cerebral lateralization and the immune system.

We attempted to confirm this hypothesis by screening for autoantibodies to DNA in the serum of mice selectively bred for degree of handedness. Serum antibody titers to double- and single-stranded DNA (DS-DNA; SS-DNA) were measured by RIA in 68 male and female mice bred for high (HI) and low (LO) handedness or from a unselected control population (CON). Antibodies to DS- and SS-DNA were significantly greater in female than in male mice. Line differences for both antibody specifities were only evident among female mice. The ranking of antibody levels by line was LO> HET> HI. Male-female differences in antibody levels (particularly against SS-DNA) were greatest in LO line animals.

The finding that autoantibody levels were inversely related to genetically-determined differences in cerebral lateralization in mice suggests an association like that noted by Geschwind & Behan (1982) in humans. Because HI and LO line mice differ in the distribution of haplotypes within the MHC (H-2) complex (Collins et al., 1985), and because HI line mice are more 'masculinized' than low line mice (Collins, 1985) our data also support the hypothesis that MHC genes and/or gonadal steroids are important in determining associations of handedness with immune function. Supported by grants NIH GM23618 and AM04761.

252.8 DECREASED NATURAL KILLER CELL ACTIVITY IN YOUNG ADULT BASAL FOREBRAIN DAMAGED AND NORMAL AGED, RHESUS MONKEYS.
L.J. Kraus, M.B. Moss and D.L. Rosene. Depts. of Neurology and Anatomy, Boston Univ. Sch. of Med., Boston, MA 02118.

A large body of literature has demonstrated the important role of neural and endocrine influences in regulating immune function. These include studies which show that damage to specific areas of the brain result in structural and functional alterations in the immune system. We report here our preliminary findings in a study examining changes in immune function following selective brain lesions in young adult monkeys compared with normal young adult and aged monkeys. As part of a multidisciplinary study of the basal forebrain and aging in the monkey, immune measures were compared with histochemical data obtained from several of the same animals described in the present study. Bilateral lesions of the basal forebrain, involving principally the substantia innominata and nucleus basalis (SI-NB), were produced by intracerebral injection of the neurotoxin, ibotenic acid. Changes in the cholinergic system were evaluated through histochemical studies of the cholinergic marker, acetylcholinesterase (AChE) and were quantified with a scanning and integrating microdensitometer. This method has been used in our laboratory to demonstrate an age-related loss of AChE in the hippocampal formation of rats and monkeys. The immune measure was natural killer cell activity (NKCA), a component of cell mediated immune function that is important in host defense against viral illness and some neoplasms as well as in maintaining immune homeostasis. NKCA is assessed in vitro by measurement of the ability of lymphocyte effectors to lyse radiolabeled NK-sensitive target cells. We assessed NKCA activity in monkeys with ibotenic acid lesions of the SI-NB (N=4), caudate nucleus (N=2) or hippocampal formation (N=1) and in young adult (4-6 yrs, N=8) and aged (25 yrs, N=4) normal rhesus monkeys. NKCA was significantly depressed in animals with SI-NB lesions as compared with young normals ($p<0.05$). NKCA in monkeys with caudate or hippocampal damage fell within the normal range. In contrast, a significant decrease in NKCA was observed in the 4 aged monkeys and was similar to the decrease seen in animals with SI-NB damage. In the 3 monkeys with SI-NB lesions assessed for AChE activity in the hippocampus, all showed marked decreases. The pattern and extent of AChE depletion was similar in two of the animals and these same two had similar levels of NKCA. The third animal had more limited depletion of AChE loss and had slightly greater NKCA. Thus, the data suggest that cholinergic loss as a consequence of lesions of the basal forebrain or of degenerative processes in normal aging may exert a marked influence on at least one important parameter (NKCA) of immune function.

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253.1 FINE STRUCTURE OF NEUROTENSIN-IMMUNOREACTIVE NEURONS IN THE CENTRAL NUCLEUS OF THE RAT AMYGDALA. S.S.W. Tay*, T.H. Williams and J.Y. Jew. Department of Anatomy, College of Medicine, University of Iowa, Iowa City, IA 52242.

An earlier immunohistochemical study (Williams, T.H. et al. In: The Amygdaloid Complex, ed. Ben-Ari, 1981) characterized distributions of several neuropeptides in the central nucleus of the rat amygdala (CNA). The heaviest concentration of neurotensin (NT)-positive reaction product was shown to be located primarily in the ventral part of the lateral subdivision, and to a lesser extent in the medial portion of the medial subdivision. The NT-immunoreactive cell bodies were localized principally in the intermediate subdivision and in the lateral part of the medial subdivision.

This light microscopic study was followed up by an electron microscopic investigation of the CNA using an avidin-biotin complex technique. Electron-dense reaction product (particles 15-25 nm in diameter) was found in cell bodies, dendrites, axons and axon terminals. In both colchicine-pretreated and untreated specimens, the perikarya and dendrites showed reaction product around profiles of rough endoplasmic reticulum, mitochondria, microtubules and vesicles, but not within their lumina. In distal dendrites, the reaction product was associated with microtubules, vesicles and postsynaptic densities.

After pretreatment with colchicine, the cytosol of NT-immunoreactive neurons contained more reaction product than the untreated material. A few NT-immunoreactive neurons showed orderly arrays of neurofilaments in their perikarya and dendrites. In cross-sections of NT-immunoreactive dendrites, the enhanced reaction product was associated with small profiles of neurotubules. In longitudinal sections of similar dendrites, the reaction product appeared to be mainly localized alongside the parallel arrays of neurotubules.

Three types of axon terminals formed synaptic contacts with NT-immunoreactive neurons in the CNA. Type A terminals contained numerous small round or oval agranular vesicles; Type B terminals were characterized by numerous pleomorphic vesicles; and Type C terminals possessed a few scattered pleomorphic vesicles. Only Type A terminals were NT-immunoreactive. All three types of terminals formed symmetrical axosomatic and asymmetrical axodendritic contacts. Type B and Type C terminals often formed synaptic glomeruli with a central NT-immunoreactive dendrite. NT-immunoreactive terminals frequently formed symmetrical axoaxonal contacts with each other as well as with non-immunoreactive Type A or Type B terminals.

This study was supported by grant NS 19578 to T.H.W.

253.2 LOCATION AND NEUROCHEMICAL CHARACTERISTICS OF NEURONS WHICH INNERVATE PENILE ERECTILE TISSUE. K. Manzanares*, N. Minorsky*, J. Kohr* and W.G. Dail. (Spon: V. Williams) Department of Anatomy, University of New Mexico, School of Medicine, Albuquerque, NM 87131.

According to classical literature, the sacral spinal cord operates through cholinergic neurons in the pelvic plexus to cause penile erection, while adrenergic neurons in the sympathetic chain prevent erection. Recent data questions whether the sacral spinal cord is the only center for penile erection and whether acetylcholine is the inhibitory substance which mediates erection. To clarify these questions, histochemical and immunohistochemical methods have been used to characterize penile neurons in the rat. A quantitative study of penile neurons, identified by dye injection into the penile crura, indicates that some 92% of the neurons in the major pelvic ganglion (MPG) are immunoreactive for vasoactive intestinal polypeptide (VIP). Ninety-five percent of the penile neurons in the MPG stain for the enzyme acetylcholinesterase (AChE), in a second group of rats. In a third group of rats, identified penile neurons in the MPG were immunostained for tyrosine hydroxylase (TH). Although TH positive principal neurons are abundant in the MPG, in a survey of over 300 neurons, none of the penile neurons stain for TH. Retrograde dye in the penile crura filled neurons in the sympathetic chain as high as the level of the diaphragm although most of the neurons occured in chain ganglia at the bifurcation of the aorta. Removal of the sympathetic chains from the diaphragm to just below the aortic bifurcation resulted in the near total elimination of adrenergic fibers in penile erectile tissue. The present study indicates that the great majority of penile neurons in the MPG are immunoreactive for VIP and have a high content of AChE. These results suggest (1) that VIP and acetylcholine may be co-localized in penile neurons in the MPG and (2) that the recently described pathway by which the hypogastric nerve innervates penile neurons in the MPG differs quantitatively rather than qualitatively from the vasodilator pathway from the sacral spinal cord. Finally, lack of staining for TH in penile neurons in the MPG and the effects of removing the chain ganglia indicate that the sympathetic chain is the major if not the sole source of the adrenergic innervation of penile erectile tissue. Supported by NIH 1R01NS19839-02 and NIH RR08139-10.

253.3 SUBSTANCE P LIKE IMMUNOREACTIVITY IN FROG SPINAL CORD. DS Adli, BM Rosenthal, RH Ho, and WLR Cruce. Neurobiology Program, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272 and Department of Anatomy, The Ohio State University, Columbus, OH 43210.

Substance P (SP) has been implicated in nociception. As a continuation of our studies on the putative roles of neuropeptides in nociceptive processing in amphibians, we examined the distribution of substance P like immunoreactivity (SPLI) in the frog spinal cord.

Adult leopard frogs (Rana pipiens) weighing 30-60gm were perfusion-fixed with Zamboni's solution. Transverse and horizontal sections of representative spinal cord levels (brachial, thoracic, and lumbar) were cut 50um thick on a vibratome or a freezing microtome. The sections were processed for the localization of SPLI by the indirect antibody peroxidase-anti-peroxidase (PAP) method of Sternberger.

Substance P like immunostaining appeared as distinct dark-brown dots and as strings of beads. These structures were interpreted to be nerve fibers and their varicosities or terminals cut in various planes of section. SPLI structures were seen throughout the gray and white matter. Small diameter SPLI fibers were seen in the dorsal root. The SPLI fibers in the ventral part of the dorsal root could be followed to Lissauer's tract. In the gray matter, dense SPLI fibers were present in an area just ventral to the central canal. In addition, a band-like SPLI region extended from the lateral edge of the gray matter to an area adjacent to the central canal. This band-like area corresponds to the region between the oval-shaped dorsal terminal field of cutaneous primary afferents and the triangular-shaped ventral terminal field of muscle primary afferents. The remaining gray matter contained a moderate density of SPLI fibers. In the white matter, SPLI fibers were densest in the dorsal part of the lateral funiculus (where Lissauer's tract is located) and the density decreased in a ventral direction. The dorsal and ventral funiculi exhibited sparse SPLI. In horizontal sections, longitudinal SPLI fibers were seen coursing in a rostrocaudal direction in the white matter. This SPLI distribution pattern is representative of all cord levels. The distribution of SPLI fibers closely matched the distribution of enkephalin like immunoreactive fibers in frog spinal cord.

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253.4 CHOLINERGIC NEURONS DEMONSTRATED BY IMMUNOHISTOCHEMISTRY IN MOUSE SPINAL CORD CULTURES. E.A. Neale, G. Bruce, E. Matthew, S.W. d'Autremont*, W.L. Strauss, L.B. Hersh and P.G. Nelson. Lab. Develop. Neurobiol., NICHD, NIH, Bethesda, MD 20205 and Dept. Biochem., Univ. Texas Health Sci. Ctr., Dallas, TX 75235

A definitive morphologic marker for a given neuron type is a valuable tool for developmental and functional studies. Such a marker for mammalian cholinergic neurons in culture has not been available. A recently developed rabbit polyclonal antibody against immuno-affinity purified human placental choline acetyltransferase (ChAT) (Bruce, G., Wainer, B.H., & Hersh, L.B., J. Neurochem., in press) was used, with the biotin-avidin peroxidase technique (Vector), to visualize neurons containing this enzyme in dissociated cell cultures prepared from 13 day fetal mouse spinal cord/dorsal root ganglia. A small percentage of neurons in these cultures exhibit immunoreactivity. Pretreatment of the cultures with colchicine is not required for somatic staining. There are no stained cells when normal rabbit serum is used instead of rabbit anti-ChAT, and staining is abolished when the antibody is adsorbed to a partially (100-fold) purified preparation of rat brain ChAT.

A few of the stained neurons in each culture are large multipolar cells with axons that can be traced from the soma for long distances and numerous axonal branches that terminate in varicosities contacting other neurons. The majority of stained cells are smaller neurons with less extensive axonal arborizations. Staining above background is not detected in one week old cultures, and stained neurons are rare after two weeks in culture. By three weeks, neuronal staining is distinct. With further development in culture, the reaction product appears more intense and the morphology of stained neurons, more complex. Immunoreactive neurons are much more numerous in cultures prepared from ventral horn (Guthrie, P.B. and Brenneman, D.B., Soc. Neurosci. Abstr. 8:233, 1984) than in those from whole spinal cord. The number and morphologic features of neurons stained using anti-ChAT are similar to those stained histochemically for acetylcholinesterase.

This antibody most likely identifies cholinergic neurons. It is unclear whether our staining conditions allow for the visualization of all cholinergic neurons in these cultures.

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253.5 EARLY CHANGES IN ACETYLCHOLINESTERASE ACTIVITY AT SYNAPTIC SITES DURING DEAFFERENTATION IN SPINAL TRIGEMINAL NUCLEUS. S.H. Broderson* and L.E. Westrum. (SPON: P.D. Swanson) Depts. of Biological Structure and Neurological Surgery, Univ. of Washington, Seattle, WA 98195.

The thiocholine method of Karnovsky and Roots is being used to investigate the light and especially the electron microscopy of the acetylcholinesterase (AChE) activity in normal spinal trigeminal nuclei (STN) and at selected times after deafferenting trigeminal rhizotomies. Trigeminal rhizotomy produces abnormal physiological activity in the STN and, after appropriate survival, even spontaneous epileptiform hyperactivity. Our intent is to correlate possible alterations in AChE activity associated with this membrane dysfunction. In normal material AChE reaction product is seen extracellularly around axons, dendrites and especially terminals, including the synaptic site. At the synaptic contact, the product may fill the synaptic cleft, but more commonly is accumulated at the presynaptic membrane, occasionally in the form of irregular columns spanning the cleft. Both classes of terminals; those with round synaptic vesicles (R) and those with flattened vesicles (F) are involved. Shortly after rhizotomy (1 day), when AChE activity appears reduced throughout, some of the degenerating R terminals show disappearance of the AChE product selectively from the synaptic cleft, although it persists in adjacent areas. By seven days the few remaining, advanced degenerated R terminals now show return of AChE activity in the cleft. Also receptor sites vacated by loss of R terminals and thus reoccupied (or reinnervated) by unaltered terminals (F usually) show AChE reaction product filling the cleft except at the "active zones" (opposite focal accumulations of presynaptic vesicles). By 14 days the synaptic neuropil shows little or no evidence of degeneration and the AChE reaction product is extensively distributed around synaptic and nonsynaptic profiles. The results indicate that early loss and subsequent increased AChE activity may partly be related to changes in the synapses during degeneration and reinnervation and that the synaptic cleft region or "active zone" is involved. The observations also provide information regarding the exact membrane associations of the AChE activity at the synapse.

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253.6 CHOLINERGIC SYSTEMS OF THE HUMAN BRAIN STUDIED BY CHOLINE ACETYLTRANSFERASE IMMUNOHISTOCHEMISTRY K. Mizukawa*, P. L. McGeer, H. Tago*, E.G. McGeer and F. Peng. Kinsmen Laboratory of Neurological Research, Dept. of Psychiatry, Univ. of British Columbia, Vancouver, B.C. V6T 1W5, Canada.

The distribution of cholinergic neurons in human brain was determined using the choline acetyltransferase (ChAT) immunohistochemical method.

Five human brains, obtained shortly after death, were removed from the cranium and perfused through the basilar and internal carotid arteries, using an electric pump, first with 1-2 liters of ice-cold phosphate-buffered saline to wash out the blood, followed by 2-3 liters of either ice-cold Zamboni's fixative or ice-cold 4% paraformaldehyde/0.35% glutaraldehyde. The brains were sliced and the slices (1-2 cm in thickness) were post-fixed in the same fixative solution as used for perfusion for approximately 24 hrs, after which they were stored in a solution of 15% sucrose in 0.1M phosphate buffer at 4$^0$C. Frozen sections (24 µm) were cut from the brain slices and stained immunohistochemically using rabbit anti-ChAT Fab fragments, a biotinylated secondary antibody and an avidin-biotin-peroxidase (ABC) marker complex.

In the forebrain, ChAT-positive cells were observed in the caudate, putamen, nucleus accumbens, nucleus basalis of Meynert, medial septal nucleus and nuclei of the diagonal band of Broca. In the brain stem, major cholinergic cell groups were found in the motor nuclei of the cranial nerves (N.III, N.IV, N.V, N.VI, N.VII, N.IX, N.X, N.XI), nucleus ambiguus, nucleus supraspinais and nuclei parabrachialis lateralis and medialis. Minor groups of ChAT-positive cells were scattered in the nucleus cuneiformis, nucleus centralis superior, nucleus vestibularis, nucleus reticularis pontis, nucleus reticularis tegmenti pontis, nucleus gigantocellularis, nucleus olivae superioris, nucleus funiculi anterioris and lateralis, nucleus gracilis and nucleus medullae oblongata centralis.

The results are compared with those previously found in the cat (Kimura et al. J. Comp. Neurol. 200:151-201,1981) and rat using similar procedures.

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253.7 CHOLINERGIC SYSTEMS OF THE HUMAN BRAIN STUDIED BY ACETYLCHOLINESTERASE HISTOCHEMISTRY. H. Tago*, P. L. McGeer K. Mizukawa* and E.G. McGeer. Kinsmen Laboratory of Neurological Research, Dept. of Psychiatry, Univ. of British Columbia, Vancouver, B.C. V6T 1W5, Canada.

A recently developed highly sensitive technique for acetylcholinesterase (AchE) has made it possible to observe clearly the distribution of this enzyme in cell bodies, dendrites and fiber tracts in human brain.

Five human brains were perfused and fixed as described in the companion abstract by Mizukawa et al. Frozen sections (24 µm) were cut from the brain slices and processed for AchE histochemistry. This method for staining free floating sections employs a peroxidase-like reactivity of products of the Karnovski and Roots' procedure in a diluted (1:100) medium. Small amounts of otherwise undetectable but highly localized reaction products are visualized by intensification using diaminobenzidine, nickel ammonium sulfate and $H_2O_2$

In control experiments, sections were incubated with eserine sulfate, DFP or BW284c51 (a specific acetylcholinesterase inhibitor). Also sections were incubated without substrate or with iso-OMPA as an inhibitor of nonspecific cholinesterase.

Careful comparison was made between neurons showing intense staining for AchE and those staining for choline acetyltransferase (ChAT) by immunohistochemistry (see companion abstract). Although AchE positive neurons were distributed in many more regions than the ChAT-positive cells, every area containing ChAT-immunoreactive neurons also contained strongly stained AchE-positive neurons. These neurons were mostly large in size (30-50 µm) and sharply outlined. They had intensely stained long processes. In a few regions (substantia nigra, locus coeruleus, hypothalamus, etc) large size neurons were found staining intensely for AchE which were completely negative for ChAT.

AChE containing axons in the cerebral cortex, hippocampus and many other regions were also observed in typical patterns of fiber tracts or in laminar arrangements.

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253.8 ANTIBODIES AGAINST HUMAN CHOLINE ACETYLTRANSFERASE: PROPERTIES AND USE FOR IMMUNOHISTOCHEMISTRY. Louis B. Hersh, Gordon Bruce and Dwight C. German, (SPON: Donnell Johns). Departments of Biochemistry, Physiology, and Psychiatry, University of Texas Health Science Center at Dallas, Dallas, Texas 75235.

The study of cholinergic neurons in human brain has become of extreme importance as these neurons appear to be selectively lost in patients with Alzheimers disease. Whereas neurons containing catecholamines or serotonin can be visualized directly using fluorescent techniques no simple method exists for visualizing neurons containing acetylcholine. Due to the non-cholinergic localization of the acetylcholine degrading enzyme acetylcholinesterase (AChE) the only specific marker for cholinergic neurons is the synthesizing enzyme choline acetyltransferase (ChAT). Over the past few years several laboratories have endeavored to purify ChAT for the production of mono-specific antisera. However, initial attempts at immunohistochemical staining in animal brains were questioned on the basis that these antibodies were not truly mono-specific. Of the 3 anti-ChAT antibodies previously used to stain human cholinergic neurones one antibody (Eng, et al. [1974] Nature 250, 243-245) has been criticised on the basis of its specificity while another antibody (Peng, et al. [1981] Neurosci. Lett. 21, 281-285) requires conversion to Fab fragments in order to achieve good staining. We report here the properties of a mono-specific anti-human ChAT antibody and its use in the immunohistochemical visualization of rat and human cholinergic neurons.

ChAT was purified to homogeneity from human placenta by immuno-affinity purification and was shown to be identical to human brain ChAT (Bruce, et al. [1985] Fed. Proc. 44, 1634). The purified human placental ChAT was used to raise polyclonal mono-specific antisera in rabbits using conventional immunization procedures. The monospecificity of the antisera was confirmed by showing its inability to react with any protein, other than ChAT, in a crude placental extract. Immunotitration of purified human placental ChAT showed the antibody to be non-inhibiting. An IgG fraction of the antibody was prepared using ion-exchange chromatography and this fraction was used for immunohistochemistry. Using standard (PAP) staining conditions cholinergic neurons were visualized in the striatium and nucleus basalis of rat brain and in the putamen and nucleus basalis of human brain. This anti-human ChAT antibody, which stains cell bodies, nerve fibres, and nerve terminals of human cholinergic neurons, will allow for further detailed studies of these neurons in normal and diseased brains.

253.9 An immunohistochemical analysis of cholinergic innervation in the cerebral cortex. F.E. Eckenstein, J. Quinn, and R.W. Baughman. Dept. of Neurobiology, Harvard Med. School, Boston, MA 02115.

Acetylcholine and other markers indicating cholinergic transmission are found in all areas of the cerebral cortex. Cholinergic cortical activity has been hypothesized to be involved in such complex mental functions as arousal and memory. Anatomical characterization of the cholinergic cortical circuitry is needed for a better understanding of the physiological role of this system.

In the rat, much of the cortical cholinergic innervation arises from large multipolar neurons in the basal forebrain. In addition intrinsic, bipolar cortical neurons also appear to be cholinergic, as judged from immunohistochemical localization of the enzyme synthesizing ACh, choline acetyltransferase (ChAT). Using immunohistochemical methods, we have analyzed the distribution of cholinergic structures throughout the cerebral cortex of the rat in detail, and have compared some of the features observed in rat with those of other species, including primates.

In rat cerebral cortex, we found the density of cholinergic terminals to be fairly constant throughout all cortical areas, except for entorhinal cortex and olfactory cortex, which show a marked increase in stained terminals. In many, but not all cortical areas, the density of ChAT-positive terminals showed a laminar distribution, and different cortical areas showed different laminar patterns of cholinergic innervation. In visual cortex, for example, a heavy band of cholinergic terminals was found at the border of layers 4 and 5; whereas layer 6 had a low density of stained terminals. Sensorimotor cortex, in contrast, had a high density of ChAT-positive terminals throughout layer 5, while layer 6 showed a moderate density. This laminar distribution of ChAT-positive terminals appears to be due to the basal forebrain projection and not to the intrinsic cholinergic cortical neurons because 1) ibotenic acid lesions of the cholinergic cell bodies in the basal forebrain abolished the laminar pattern of terminal staining in cortex and 2) terminals of the intrinsic cortical VIP/ChAT cells, visualized by staining for VIP, do not have a laminar distribution. The cholinergic basal forebrain projection to cortex was found in all species so far studied. In contrast, ChAT-positive bipolar cortical neurons were present only in rat and mouse, where they also contained vasoactive intestinal polypeptide (VIP). In rabbit, guinea pig and cat, bipolar VIP-positive neurons were present in the cerebral cortex, but these neurons did not appear to contain ChAT. Neither ChAT- nor VIP-positive neurons were found in the macaque cerebral cortex. (NIH EY03502 and ADRDA 84-1468)

253.10 Ultrastructural characterization of choline acetyltransferase containing neurons in the nucleus tractus solitrii of rat brain. D.M. Armstrong, A. Rottler* and V.M. Pickel. Dept. of Neurosciences, UCSD, LaJolla CA. 92093 & (VMP) Laboratory of Neurobiology, Dept. of Neurology, Cornell University Medical College, New York, NY 10021.

In the present study we employed the highly sensitive double peroxidase-antiperoxidase method in order to determine the distribution and ultrastructural morphology of choline acetyltransferase (ChAT) positive neurons in the nucleus tractus solitarii (NTS) of rat. ChAT is the rate limiting enzyme in the production of acetylcholine and is regarded as the most specific marker of the acetylcholine neurotransmitter system.

A well characterized antibody to ChAT (Eckenstein F. & Thoenen, H., (1982) EMBO J. 1, 363-368) was localized by immunocytochemistry in 50um Vibratome sections of rat brain fixed by vascular perfusion with a mixture of 4% paraformaldehyde and 0.2% glutaraldehyde in 0.1 M phosphate buffer.

ChAT immunoreactivity was localized to the cytoplasm of selectively labeled neuronal perikarya and dendrites. Most of the dendrites arose from ChAT-labeled neurons within the ventrally adjacent dorsal motor nucleus of the vagus (DMX). Within the NTS immunolabeled perikarya were relatively few in number, and for the most part were distributed within the caudal 2/3 of the nucleus. These neurons were only moderately labeled when compared to the ChAT-positive neurons in the DMX or hypoglossal nucleus. The labeled perikarya were typically oval and gave rise to 1 - 3 proximal processes.

As suggested by light microscopy the ultrastructural analysis of the NTS demonstrates ChAT immunoreactivity within a few perikaryon and numerous dendrites. In both instances the peroxidase reaction product was distributed throughout the cytoplasm with some selective accumulation on microtubules or around the outer rim of mitochondria. The plasma membrane of these immunolabeled neurons and dendrites often formed synapses with unlabeled presynaptic axon terminals. Although the chemical identity of these unlabeled boutons remains to be determined it is likely that at least some of them arise from peripheral afferents containing one of any number of neuropeptides (eg. substance P or enkephalin). In addition to forming classic synaptic contacts, many immunolabeled dendrites were observed in direct apposition to the basal lamina of blood vessels. Cholinergic processes have been observed innervating blood vessels within other brain regions, and thus our data indicate that a cholinergic mechanism may be important in the regulation of the cerebral microvasculature throughout many regions of the central nervous system.

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253.11 THE ULTRASTRUCTURAL LOCALIZATION OF GABA-LIKE IMMUNOREACTIVITY IN THE NUCLEUS SOLITARIUS. B.E. MALEY AND K.A. HOWES*. Dept. of Anatomy, Univ. Kentucky Med. Sch., Lexington, KY 40536.

The nucleus solitarius is involved in the central regulation of cardiovascular functions. GABA, an inhibitory neurotransmitter, plays a role in this regulation, although the morphological basis for its action is not presently understood. The present study was undertaken to investigate the synaptic circuitry involving the GABA system in the nucleus solitarius.

Following fixation with a solution of 4% paraformaldehyde and 0.3% glutaraldhyde in phosphate buffer, 75 um sections of the nucleus solitarius were cut on a vibratome, incubated in GABA antiserum and processed with the peroxidase, antiperoxidase technique. The sections then were osmicated, dehydrated and embedded in Spurr's resin.

Examination of thin sections revealed GABA immunoreactivity within synaptic terminals, dendrites and neuronal cell bodies of the nucleus solitarius. The reaction product was generally present adjacent to the cytoplasmic side of membrane bound structures as well as to the membranes of the synaptic vesicles. GABA synaptic terminals, which contained numerous small clear vesicles, were distributed along the dendritic tree as well as the cell soma. The GABA terminals synapsed mainly on small to medium sized dendrites, although significant numbers of contacts were also present on large dendrites and cell bodies. On rare instances, GABA terminals were presynaptic to unlabelled axona. In addition to GABA terminals contacting unlabelled neuronal elements, GABA terminals also synapsed upon GABA immunolabelled dendrites and cell bodies. GABA immunolabelled neurons measured 8-15 um in diameter with the reaction product mainly restricted to membrane bound structures within the perikaryal cytoplasm. The GABA immunostained neurons were postsynaptic to both unlabelled and GABA immunolabelled synaptic terminals. Dendrites containing GABA immunoreactivity were characterized by reaction product associated with their microtubules, and they were postsynaptic to both labelled and unlabelled synaptic boutons.

The localization of GABA immunoreactivity in neuronal structures not only provides additional confirmation of a role for GABA in neural transmission, but also suggests a possible physiological function in the nucleus solitarius. Additionally, the presence of GABA-GABA synaptic contacts indicates a possible self-modulatory role for the GABA system. Supported by NIH HL 30702 to B.E.M.

253.12 IMMUNOCYTOCHEMICAL LOCALIZATION OF GABA IN DIENCEPHALIC AND MESENCEPHALIC NUCLEI OF THE CHICK BRAIN. W. J. Crossland and R. H. Granda*. Dept. of Anatomy, Wayne State Univ. Sch. of Med., Detroit, MI 48201.

We have identified cell bodies and axonal pathways in the chick brain using a polyclonal antibody to gamma aminobutyric acid (GABA) (Immunonuclear Corp.) visualized with an avidin-biotin-horseradish peroxidase complex (Vector Labs). All tissue sections were preincubated to remove endogenous peroxidase activity. Omitting the primary antibody abolished all stainable activity in the sections. Moreover, the antibody stained cells and presumed axonal processes in the molecular layer of the cerebellum, an observation consistent with the known morphology and distribution of GABA-containing stellate and basket cells.

There were two patterns of distribution of GABA positive cell bodies. (1) A group of nuclei containing predominately small to medium-sized cells which receive direct retinal input: lateralis anterior, ventral lateral geniculate, dorsolateralis anterior pars lateralis, ectomamillary, lentiformis mesencephali, and the stratum griseum et fibrosum superficiale of the optic tectum (primarily sublaminae a, c, and i). (2) A group of nuclei containing large and medium-sized cells which formed a band extending from the rostral thalamus to the caudal optic tectum, respectively: superior reticular nucleus pars dorsalis and ventralis, the posteroventral nucleus (tentative identification), the nucleus subpretectalis, and most caudally, the nucleus isthmi pars magnocellularis. In addition, GABA-positive cells were found in the nucleus mesencephalicus lateralis pars dorsalis of the midbrain, the preoptic area, the red nucleus, and scattered in the midbrain reticular formation. Some cells within the optic tract near the ventral lateral geniculate were also labeled.

Although many of the large cells are almost unquestionably neurons, the identity of the smaller cells, particularly those found in the optic tract, is not clear. Furthermore, we have no evidence as yet that any of these cells in fact use GABA as a transmitter, although from what is known of the avian brain, and by analogy with the mammalian brain, it seems likely that GABA may play such a role.

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253.13 IMMUNOHISTOCHEMICAL LOCALIZATION OF GLUTAMATE AND GLUTAMINASE IN NEURONS OF THE PONTINE NUCLEI AND ASPARTATE AND AATASE IN NEURONS OF THE INFERIOR OLIVE. A.J. Beitz, A.A. Larson, J.E. Madl*, R.A. Altschuler and M.M. Mullett*. Univ. of Minnesota, St. Paul, MN 55108 and NINCDS, Bethesda, MD 20205.

Glutamate and aspartate are putative, excitatory amino acid neurotransmitters in the central nervous system. Both glutaminase, an enzyme which converts glutamine to glutamate, and aspartate aminotransferase, an enzyme which is involved in the interconversion between glutamate and aspartate, have been proposed as markers of neurons which use excitatory amino acids as neurotransmitters. Aspartate has been proposed as the transmitter of the olivocerebellar system, while the transmitter of the pontocerebellar system is unknown. The present study utilized a monoclonal antibody against carbodimiide-fixed glutamate (CFG), a monoclonal antibody against carbodiimide-fixed aspartate (CFA) and polyclonal antisera against glutaminase and aspartate aminotransferase (AATase) to analyze the distribution of excitatory amino acids in the pontine nuclei and inferior olivary nucleus. Five rats were perfused with a solution of 5% carbodimiide and 0.5% glutaraldehyde followed by 5% glutaraldehyde for use with the monoclonal antibodies. Five additional animals were perfused with a solution of 4% paraformaldehyde and 0.1% glutaraldehyde for use with the glutaminase and AATase antisera. Fifty micrometer thick vibratome sections were cut and reacted with the primary antisera. Sections were then immunostained using the avidin-biotinylated peroxidase procedure in conjunction with diaminobenzidine and hydrogen peroxide. CFG-like, AATase-like and glutaminase-like immunoreactivity were found in neurons in all subdivisions of the pontine nuclei. Following HRP injections into the cerebellum 81% of retrogradely labelled neurons in the basilar pontine nuclei were double-labelled with CFG immunoreactivity. Both CFA-like and AATase-like immunoreactivities were evident in neurons of the inferior olive. HRP injections into the cerebellum indicated that retrogradely labelled olivary neurons also contained aspartate immunoreactivity. These results suggest that glutamate may be the major neurotransmitter of pontocerebellar fibers, while aspartate is associated with the olivocerebellar projection. Supported by NIH grants NS19208 and DE06682 to A.J.B. and NIH grant NS17407 to A.A.L.

253.14 IMMUNOCYTOCHEMICAL LOCALIZATION OF GLUTAMATE AND ASPARTATE IN THE RETINA OF THE RAT. J.E. Madl*, A.A. Larson, A.J. Beitz and R.L. Johnson*. (SPON: D. Brown) Univ. of Minnesota, St. Paul, MN 55108.

Glutamate and aspartate are putative, excitatory amino acid neurotransmitters in the retinas of vertebrates. Biochemical studies employing microdissection have found highest concentrations of these two amino acids in the inner plexiform layer (IPL) and ganglionic cell layer (GCL) of the retina. The present study utilizes monoclonal antibodies with high degrees of specificity for fixative-treated glutamate and aspartate to analyze the distribution of excitatory amino acids in the retina of the rat. In agreement with the microdissection studies, the most intense immunocytochemical staining of the retina for both aspartate and glutamate was found in the IPL and GCL. Intense staining was also seen in certain cell bodies of the inner nuclear layer (INL). Hybridomas producing monoclonal antibodies were made by fusing myeloma cells with spleen cells from Balb/c/umc mice immunized with gamma-L-glutamyl-L-glutamate (gamma-Glu-Glu) or beta-L-aspartyl-L-aspartate (beta-Asp-Asp) conjugated to keyhole limpet hemocyanin (KLH) using glutaraldehyde-borohydride. Monoclonal antibodies obtained from mice immunized with beta-Asp-Asp/KLH gave highest intensity staining of the IPL, GCL, and some cell bodies in the INL of the rat retina using an indirect immunoperoxidase technique. Similar but less intense staining of these same layers was seen using a monoclonal antibody obtained from the mice immunized with gamma-Glu-Glu-/KLH. Specificity of these monoclonal antibodies was determined by the ability of small molecules to inhibit immunocytochemical staining of the retina and by the ability of the small molecules to inhibit enzyme-linked immunoassay (ELISA) reactivity of the monoclonal antibodies for the conjugates with which the mice were immunized. One monoclonal antibody was found to react strongly on competitive inhibition ELISA with gamma-Glu-Glu and other small molecules containing glutamic acid but not with similar small molecules containing aspartate, GABA, or glutamine. Two monoclonal antibodies were found to react strongly in the competitive inhibition ELISA assay with beta-Asp-Asp and other aspartate containing small molecules but not with similar small molecules containing glutamate or GABA. Immunocytochemical staining of the retina was inhibited by the same small molecules that reacted strongly with the monoclonal antibodies in the competitive inhibition ELISA assays. Supported by NIH grants NS07367 to J.E.M., NS17407 to A.A.L. and DE06682 to A.J.B. and also NSF grant BNS 83-11214 to A.J.B.

## NEURAL BASIS OF BEHAVIOR: INTERHEMISPHERIC RELATIONS I

254.1 HEMISPHERIC ASYMMETRIES FOR A VISUAL SEARCH TASK. Jeffrey D. Lewine* and Garth Elias*. (Spons. R.W. Doty) Center for Brain Research, University of Rochester, Rochester, New York 14642.

It has often been argued that the left cerebral hemisphere acts as an analytical/serial processor while the right serves as an holistic/parallel processor. Surprisingly, few laterality studies have used tasks specifically designed to differentiate these two information processing strategies.

A visual display search task was used to access the information processing schemes of the two hemispheres. Subjects were given a single target letter to remember at the beginning of each trial. A visual probe display of 1,2,4, or 6 letters arranged vertically was then presented with equal probability $2^{\circ}$ to the left (LVF), or right (RVF) of fixation, or directly across the fixation point, for 150 msec. The subject's task was to indicate if the target letter was present or absent from the display. Ten right-handed subjects were each given a total of 1152 trials across two experimental sessions.

If visual search proceeds in a serial fashion, response time will be related to the number of items being examined. In contrast, if search proceeds in an holistic/parallel fashion, then response time should be independent of the number of display elements.

A regression analysis relating response time to the number of elements in the display was performed for each visual field of presentation x target present/absent grouping. The obtained equations were as follows:

LVF/target-present: y=453+14.9x LVF/target-absent: y=461+33x
RVF/target-present: y=484+3.0x RVF/target-absent: y=484+27x
(y=response time in msec; x=number of letters in the display)

The data obtained from LVF presentations clearly suggest the use of a serial self-terminating search strategy. In contrast, the negligible slope (3.0x) for RVF/target-present trials is indicative of a parallel search. Interestingly, the slope for RVF/target-absent trials is similar to that of LVF/target-absent trials. It is perhaps the case that when the left hemisphere fails to find the target letter present in the display, a response is delayed until the right hemisphere confirms this. The observed response-time function is thus generated by the serially processing right hemisphere despite a RVF presentation.

In summary, the two hemispheres may indeed use different information processing strategies but, in the present situation, it seems to be the right hemisphere that serves as a serial processor while the left searches this alphabetic material in an holistic fashion. Experiments are underway to explore how search strategies are affected by the use of non-linguistic stimulus materials. Also, experiments exploring the search schemes of left-handed subjects are being conducted.

254.2 TASK-INDUCED DIFFERENTIAL CORTICAL ACTIVATION PATTERNS. M.N. Mitrushina* and J.S. Stamm. Dept. of Psychology, SUNY @ Stony Brook, New York 11794.

Measures of task-dependent cortical activations were assessed by bilateral EEG recordings from frontal, temporal, parietal and occipital areas. In a preliminary study 16 cognitive tests were administered to 200 adults and factor loadings determined. Two pictorial tests, the Raven Progressive Matrices and the Space Relations Test,had the most divergent loadings on Verbal and Spatial factors, respectively. They were used in the main experiment for EEG recordings during problem solving by 22 right-handed males. Recordings were obtained for 20 trials of each task and 16-sec. trial epochs were subjected to Fast Fourier analyses. The resulting averaged intensity values for conventional EEG spectral bands and characteristic frequencies for the alpha band (8 to 13 Hz) were subjected to statistical analyses. Indices of cortical activation were compared between verbal and spatial tasks for all subjects and between subgroups of verbalizers and visualizers. Each subject was assigned to one subgroup on the basis of his performance index, derived from response speed and accuracy on the two tasks.

Results are: (1) The most pronounced EEG discriminators between the two performance subgroups are the left and right parietal and the right frontal areas; (2) The left parietal zone provides the most pronounced discrimination between the two subgroups and its major involvement is presumed to be associated with the sequential cognitive mode; (3) There were significant interactions between the left parietal and the right frontal regions; (4) The two parietal areas show characteristic frequency shifts in opposite direction for the task conditions. Corresponding activation patterns were not revealed by any other pair of homologous areas. The findings imply complex interactions among the two parietal and right frontal areas, associated with sequential and holistic strategies.

The present findings are consistent with: (1) Anatomical evidence of interconnections between inferior parietal and premotor regions; (2) The attentional-arousal model of information processing, emphasizing distinct functional specialization of the left and right parietal areas and a mediating role of the right frontal area in processing of incoming stimuli. Further research on task-dependent EEG measures should consider subjectively preferred cognitive strategies which, together with objective task demands, influence processes for problem solving and accompanying neuronal changes.

254.3 LEFT AND RIGHT HEMISPHERE CONTRIBUTIONS TO A MOTOR SEQUENCING TASK. B. Sivak* and C. L. MacKenzie (SPON:G. Renninger). Dept. Of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1.

The study investigates left and right hemisphere contributions in a rapid finger sequencing task. The existence of a sequencing control center in the left hemisphere is a prime interest (Kimura, 1977, 1982).

Sensory input was lateralized for 16 right-handed subjects to either the right or left hemisphere by using specially designed contact lenses which restricted input to either the left visual field of the left eye or the right visual field of the right eye. The non-participating eye was occluded. A control condition involved subjects using both eyes during testing. Subjects performed a sequence of two keypresses in response to a light stimulus with either the right or left hand in a choice reaction time paradigm. Two experimental conditions were used. In Condition A, subjects always knew which hand to use but did not know which sequence to perform. In Condition B, subjects always knew which sequence to perform but did not know which hand to use.

Reaction time results indicate that there are no significant differences between the left and right hemisphere in selecting a sequential response when there was sequence uncertainty but hand certainty nor when there was sequence certainty but hand uncertainty. Reaction time was significantly slower when subjects wore the contact lens during testing. Further, reaction time was significantly faster for all stimulus-response combinations in Condition B when compared with all the stimulus-response combinations in Condition A. Overall, subjects made fewer errors in Condition B than in Condition A.

Interfinger time results show left eye-left hand advantage when there is sequence uncertainty but hand certainty and left eye-right hand and left eye-left hand disadvantage when there was sequence certainty but hand uncertainty.

The reaction time results do not support the notion of a control center in the left hemisphere for selection of components of a two element sequential keypress prior to movement initiation for both hands. However, the interfinger time results indicate that there are differences in the processing ability of the left and right hemispheres in a sequencing task after movement initiation.

254.4 THE RELATIONSHIP BETWEEN INPUT INTERFERENCE AND OUTPUT INTERFERENCE MEASURES OF LANGUAGE LATERALIZATION. Richard S. Lewis, Donna L. Orsini and Paul Satz*. Neuropsychiatric Inst., UCLA, Los Angeles, CA 90024.

Various experimental procedures have been used to infer hemispheric language representation in normal individuals. These methods include input interference tasks, such as dichotic listening and visual half-field, and output interference tasks,such as concurrent finger tapping and speaking. Kinsbourne [In J.B. Hellige's (Ed.) Cerebral Hemisphere Asymmetry, 1983] has argued that output interference tasks are more advantageous measures of laterality because output processes are more lateralized, more observable, less susceptible to attentional shifts, and less likely to yield facilitory effects. The present study investigates the relationship between input interference and output interference measures of cerebral lateralization, and their clinical utility, in a large sample of normal subjects.

One input interference laterality task, dichotic listening, and two output laterality tasks, concurrent finger tapping and speaking (dual manual-speech vocalization), and concurrent finger tapping and reading (dual manual-reading), were administered to 300 left-handed and 255 right-handed subjects (see Orsini et al., Neuropsychologia, 23, 223-232, for procedural details).

The results revealed low correlations between the input interference and output interference tasks (r = .11 for the correlation between dichotic listening and the dual manual-speech vocalization task; r = .06 for the correlation between dichotic listening and the dual manual-reading task). Furthermore, the results obtained with the dichotic listening technique more closely approximate the findings reported using the Wada and incidence of aphasia methods than either of the dual task measures. The proportion of right-handed subjects demonstrating findings suggestive of left hemisphere language dominance was 88% on dichotic listening, 63% on the dual manual-speech vocalization task, and 57% on the dual manual-reading task; for left-handers it was 76% on dichotic listening, 43% on the dual manual-speech vocalization task, and 42% on the dual manual-reading task.

These results will be discussed with reference to Kinsbourne's functional distance model (Kinsbourne, 1983) and their utility for predicting language lateralization.

254.5 VISUAL EVOKED RESPONSE ESTIMATES OF INTERHEMISPHERIC TRANSFER TIME IN HUMANS: OCCIPITAL REGION EFFECTS C. Saron*, P. D. Reiner* and R. J. Davidson* (SPON: R. Halperin) Dept. of Psychology, University of Wisconsin, Madison, WI 53706 and Dept. of Psych. SUNY Purchase, Purchase, NY 10577

Interhemispheric transfer time [IHTT] has been estimated by examining time differences between ipsilateral and contralateral responses to unilateral stimuli. Electrophysiological studies of IHTT have used evoked potential peak latency shifts and cross-lag correlations between waveforms from homologous sites as their estimates of IHTT. The purpose of this study was to compare these approaches and to examine differences in IHTT estimates recorded from two different occiptal scalp regions.

Visual evoked potentials [VEP's] to hemiretinal stimuli during a reaction time task were recorded from 12, 20 to 30 year old, right-handed males. 3.6° [V] by 3.0° [H] checkerboards were presented 2.9° to the left or right of central fixation for 10 ms with an ISI of 1.5-3.5 sec. 100 stimuli were presented to one visual field at a time. Subjects lifted their left index finger in response to each stimulus.

VEP's were derived by averaging EEG recorded from scalp sites O1, O2, O3 and O4 (2cm lateral to O1 and O2), referred to linked ears, for 200 ms following the onset of each stimulus. EOG was recorded from the external canthi, and from supraorbit to suborbit. Epochs confounded with eye movement were not averaged.

VEP latency differences between sites within a visual field [LVF or RVF], were examined separately for each region [O1/O2 or O3/O4], using a lag to maximum correlation procedure and measurements of P100 peak latencies. For the correlation procedure, a 50 ms wide window on the waveform from the contralateral pathway, was lagged 30 ms in both directions against the other waveform in 0.4 ms steps. The zero lag correlation [Or], maximum correlation [Mr] and its associated lag time were computed. This procedure was repeated 32 times with the window start position [W] increasing by 2 ms from 50 to 114 ms. The window from which data were chosen for further analysis represented the first peak in the function Mr vs. W for all W's whose Or > -0.5, Mr > 0.75 and Mr - Or > 0.05. If no peak was found, data from the criteria-meeting window with the greatest Mr was used. Peak latency measures were obtained by measuring the latency of the greatest positivity between 90 and 140 ms. Estimates of IHTT were computed by subtracting the latency of the peak from the contralateral waveform from the corresponding peak recorded from the homologous ipsilateral site.

Lag and peak latency differences were examined separately, with ANOVAs using visual field and region as factors. For both the lag and peak latency data, the ANOVA revealed no significant differences between visual fields for either region. For the lag data, a significant region effect was obtained [F(1,11)=18.11,p=.0014]. The mean (SD) lag values, across visual fields, were: O1/O2 = 7.0 (7.2) ms, [Or = 0.84 (0.09), Mr = 0.95 (.04)]; O3/O4 = 13.1 (6.7) ms, [Or = 0.51 (.44), Mr = 0.95 (0.05)]. For the latency data, a significant region effect was also obtained [F(1,11)=9.56,p=.01]. The mean (SD) peak latency differences, across visual fields were: O1/O2 = 7.9 (9.3) ms, O3/O4 = 14.5 (10.2) ms. Mean (SD) P100 peak latencies by region, averaged across visual field and hemisphere were: O1/O2 = 114.9 (6.9) ms, O3/O4 = 112.8 (5.9) ms.

These data indicate that relatively small changes in electrode sites play an important role in interpreting VEP esitimates of IHTT. However, markedly different approaches to waveform quantification have revealed similar effects. This work was supported by the March of Dimes Birth Defects Foundation and an equipment loan from Teca Corporation.

254.6 INTERHEMISPHERIC TRANSFER EXAMINED THROUGH SIMPLE REACTIONS TO LATERALIZED FLASHES OF LIGHT IN COMMISSUROROMIZED PATIENTS. J. Sergent. Montreal Neurological Institute, and Department of Psychology, McGill University, Montreal, PQ, H3A 2B4, Canada.

Simple reactions to lateralized flashes of light suggest that transmission of information between the two cerebral hemispheres is achieved at very high speed via the corpus callosum. The nature and efficiency of this transmission, however, vary as a function of the response modality, and a manual response does not yield the same pattern of results as a verbal response (Tassinari et al., Human Neurob., 2:77-85, 1983). The source of this discrepancy was further examined in complete forebrain commissurotomy patients who had to produce, in three separate experiments, either a finger, a blowing, or a verbal response to a 10-ms flash of light appearing in either the left or the right visual field. A control subject was tested in exactly the same experimental conditions, and his results conformed to the pattern typical of such experiments.

For the two patients (L.B. and N.G.), a blowing response was made equally fast to left and right field presentation, indicating that the two hemispheres of each patient could process the light equally efficiently. The finger task yielded a highly significant interaction between visual field and responding hand, and the reaction time difference between ipsilateral and contralateral responses was of the order of 30 and 50 ms for L.B. and N.G., respectively, suggesting that subcortical transfer of information is highly inefficient in the manual task compared to a transmission time of 2-3 ms through a callosal route. By contrast, the verbal task resulted in no visual field difference, even though the specialized mechanisms of the left hemisphere are required for speech production.

The results confirm that different pathways may subserve transmission of information across the hemispheres and they further suggest that a verbal response may be initiated in subcortical structures to which both hemispheres have equally efficient access before being organized within the left hemisphere.

254.7 RIGHT/LEFT PROCESSING OF PERSPECTIVE CUES FOR VISUAL DISTANCE IN COMMISSUROTOMY SUBJECTS. A. Cronin-Golomb* (SPON: B. Vermeire). Div. of Biology, Calif. Inst. of Technology, Pasadena, CA 91125

In normal subjects, a dot is perceived as smaller than its actual size if presented to the top half of a field, and larger than actual size if presented to the bottom half of the field. Four complete commissurotomy subjects (LB, NG, AA, and RY) were examined on this task. Stimuli were flashed for 150 msec to one or the other visual hemi-field, and included solid dots of three sizes corresponding to 0.3°, 0.8°, and 1.3° of visual field. Any one dot appeared in one of three positions (top, middle, or bottom) on a 6 X 7° card. Subjects were to point to that dot of the nine-choice array in free vision that was of the same size and position as the sample dot. Right hemisphere errors were found to lie in the direction of the normal illusion (chi square, $p < .01$). The effect was enhanced when a 'natural' gradient background (a plane of horizontal lines, receding toward the top of the card) was used, rather than a plain white background. By contrast, left hemisphere errors were generally perceived as smaller in the bottom and larger in the top half of the field ($p < .01$). No significant effect of background was observed.

Further, the right hemisphere of three subjects (LB, NG, and AA) more often chose the correct answer when the stimulus card represented a relatively 'natural' distance relationship (e.g., small dot at the top) than when the relationship was relatively 'unnatural' (e.g., small dot at the bottom.) The opposite effect was obtained for the left hemisphere in subjects NG and RY, for whom left hemisphere performance was better on trials involving 'unnatural' than 'natural' relationships. For both hemispheres, the results were independent of background used.

The findings suggest that the separated hemispheres may be analyzing some visual perspective cues in different ways. The right hemisphere appears to use natural perspective cues to determine the actual size and position of an object. When it does make an error, it judges objects high in the field as small, and those low in the field as large, again corresponding to the rules of constancy scaling. This result implicates the right hemisphere in the processing of perspective cues, which may in turn explain the observed right hemisphere susceptibility to those optical illusions such as the Ponzo that have been described in terms of perspective relations. The left hemisphere, on the other hand, may be using its skills as a feature extractor to seek out and process significant details in a field, such as usual perspective cues that contradict rather than conform to the rules of constancy scaling.

254.8 VISUALLY GUIDED REACHING AND GRASPING IN PATIENTS WITH UNILATERAL BRAIN DAMAGE. M. A. Goodale, M. E. Cohen*, J. D. Fisk*, and C. Routhier* (SPON: A. J. Hudson). Department of Psychology, University of Western Ontario, London, Ontario, Canada, N6A 5C2.

The spatiotemporal organization of visually guided reaching and grasping movements was studied in patients with restricted unilateral damage of either the right or the left hemisphere. All the patients were normally right handed. Strobe-shutter videotape records were made of the limb and hand as the subject reached toward and touched a small visual target (0.25 degrees) that was presented on a tangent screen either 10 or 20 degrees to the right or left of fixation. The reaction times and kinematics of the reaching movements were extracted from the videotapes by means of a computer-assisted digitizing system. All comparisons between the patient groups and control subjects were made with the hand ipsilateral to the lesion site. While patients with left-hemisphere lesions took no longer to initiate a pointing movement than age-matched control subjects, patients with right-hemisphere lesions took significantly longer than either the control subjects or patients with left-hemisphere lesions to initiate the same sort of movement. Once a movement was initiated, however, the performance of the right-hemisphere group differed little from that of the control group. In contrast, patients with left-hemisphere lesions took much longer to complete their pointing movement than either of the other two groups. The increase in the duration of the movement in the left-hemisphere patients was primarily due to prolonged period of deceleration during the final approach to the target. Both patient groups made larger terminal errors than the control group. In a second task, in which subjects were required to reach toward and grasp an object that differed in shape but not in location from trial to trial, the patients with right-hemisphere lesions took no longer than control subjects to initiate the reaching movement but took slightly longer to complete the entire sequence. Left-hemisphere patients, however, were slower both to initiate and to complete the reaching movement. These results suggest (1) that mechanisms within the right hemisphere may play an important role in determining the direction in which a particular movement should be made and (2) mechanisms within the left hemisphere participate in the selection of the appropriate configuration of motor programs necessary to achieve that particular movement.

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254.9 DISSOCIATION BETWEEN AUDITORY AND VISUAL CHOICE REACTION TIME IN THE TWO HEMISPHERES UNDER THE INFLUENCE OF ALCOHOL: E. Pöppel, Th. Steinbach*, J. Janzen* and W. Spann*. Institut für Medizinische Psychologie and Institut für Rechtsmedizin, Ludwig-Maximilians-Universität, 8 München 2, F R Germany

Alcohol appears to have a selective influence on the two hemispheres. This can be concluded on the basis of experiments in which intermodal choice reaction time (CRT) was determined. In these experiments, visual, auditory and tactile stimuli were presented in random order and the subject had to press as fast as possible one of the three appropriate buttons. Stimuli were lateralized in such a way that only (or predominantly in case of the auditory and tactile modality) the left or the right hemisphere was stimulated. Twelve male subjects participated in each of the two experiments. In case the left/right hemisphere was stimulated subjects had to use their right/left index finger for reaction. Thus, stimulation and initiation of motor response were controlled by the same hemisphere. After a baseline measurement in which at least 10 single reaction times for each modality were obtained, subjects drank beer and hard liquor in order to raise the alcohol level of the blood to a predetermined level. Blood tests showed that the left hemisphere group reached a level of 0.141 % and the right hemisphere group of 0.136 % . Then the CRT-experiment was repeated. With alcohol, tactile CRT was increased for the two hemisphere groups. With alcohol, auditory CRT was increased significantly only for the left hemisphere group. With alcohol, visual CRT was increased significantly only for the right hemisphere group. The same holds true if instead of reaction time the standard deviations of CRT are considered. In a control experiment it could be shown that auditory simple reaction time is increased both for the left and the right hemisphere group. In can be concluded that the interhemispheric dissociation between auditory and visual CRT is not due to a general slowing down of sensory processing and motor response, but that the dissociation appears to be dependent on a modality-specific influence of alcohol on a higher-order choice process.

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254.10 ANALYSIS OF EEG COHERENCE DURING SPONTANEOUS RESPIRATORY SUSPENSION. A.T. Arenander, B. Lou*, W. Sheppard*, W.L. VanZandt, R.S. Hernandez and D. Krieger*. Neuroscience of Human Consciousness Research Laboratory, Maharishi International University, Fairfield, IA 52556.

Previous studies have reported a unique configuration of physiological and psychological processes which emerge during the practice of the Transcendental Meditation technique that correspond to a state of awareness without thoughts or spatio-temporal boundaries. In some subjects, this state of transcendental consciousness has been consistently correlated with extended periods (~40 sec.) of spontaneous respiratory suspension (SRS) which are separated by periods of awareness with thoughts and shallow respiratory activity (RA). We report here a detailed analysis of the EEG brain wave dynamics during RA and SRS. Our results support the concept that brain activity can display a highly coherent and simple state of functioning.

Data were obtained from a 10 lead montage, and analyzed for power and phase coherence parameters for the following frequency bands: theta, alpha 1, alpha 2, and beta 1. During the meditation period, relative fluctuations in power were similar across all bands and all leads. High ($>.90$) intrahemispheric coherence was found, with left hemisphere (LH) coherence greater than right (RH). Intrahemispheric coherence values were lower ($<.70$). Also, intrahemispheric coherence values were relatively constant regardless of the distance between scalp locations. Onset of SRS was associated with sharp increases in coherence values, with both hemispheres reaching nearly identical ceiling values.

Principal factor analysis of the coherence matrices showed that the factor structure changed with the onset of SRS, indicating simplification of EEG dynamics: 2 significant factors were typically obtained where factor 1 (LH) and factor 2 (RH) shared equal variance, all leads individually loaded $>0.9$, and the communality reached greater than 90%. Finally, complex demodulation at peak alpha 1 frequency produced nearly identical patterns of amplitude and phase fluctuations in bilateral leads during meditation. During SRS, phase activity stabilized. These results indicate that the human brain is capable of displaying a simple, stable state of neurophysiological functioning characterized by a high degree of sustained spatial and temporal integration.

254.11 LATERALIZED MEMORY DEFICITS IN PATIENTS WITH MAJOR PSYCHOPATHOLOGY COMPARED TO UNILATERAL STROKE PATIENTS. C.E. Naylor and F.B. Wood*. Section of Neuropsychology, Bowman Gray Sch. of Med., Winston-Salem, NC 27103

Based on previous research demonstrating lateralized deficits in patients with major psychopathology (see reviews; Flor-Henry, P., Ann. of N.Y. Acad. of Sci., 280: 777, 1976; Wexler, B.E., Am. J. of Psychiat., 137: 279, 1980; and Tucker, D.M., Psychol. Bull., 89, 19, 1981), patients with major depression (Dep.,N=15) and schizophrenia (Schiz.,N=15) were compared to two groups of stroke patients (25 right hemisphere lesioned (RH) patients and 40 left hemisphere lesioned (LH) patients), as well as to a sample of normals (N=50), matched for age, sex, and education. An equal distribution of frontal, central and posterior lesions was represented.

All subjects completed a series of memory tests as well as two "non-memory" tests, one visual (Porteus Mazes-PM) and one verbal (verbal fluency-FAS test). The two verbal memory tests consisted of delayed recall of a prose passage (cowboy story) and the post-distractional free recall trial of the Rey Auditory Verbal Learning Test (RAVLT). The two visual memory tests were delayed recall of a complex geometric design (Rey-Taylor figure) and a Figural Learning Test devised by us to constitute a nonverbal analogy to the RAVLT. It consists of five rehearsal trials of a list of novel shapes followed by a post-distractional free recall trial. Other tests of neuropsychological functioning were inserted to provide approximately a one hour delay between recalls.

The profile of the stroke patients revealed the expected cross-over interaction on all 6 tests, i.e. the RH patients were worse than LH patients on visual tasks while the LH patients were worse on verbal tests. The comparisons between the two lesion groups reached statistical significance on all tests except PM, which showed a similar though non-significant trend for RH patients to do worse. Both lesion groups were significantly worse than control subjects on all 6 tests indicating a generalized deficit as well.

Schiz. patients showed a profile that was statistically indistinguishable from LH patients on the four memory tests (worse than dep. on verbal memory tests) while dep. patients were statistically identical to the RH group (worse than schiz. on visual memory tests). Although the same cross-over interaction was found for the memory tests, the psychopathology groups did not differ on other tests, performing better than lesion groups. In conclusion, the commonality between schiz. and LH patients and between dep. and RH patients was observed most clearly on tests of memory functioning. The pattern of deficits in psychopathology is most similar to those found secondary to unilateral lesions which are centrally located on the anterior-posterior axis. These results suggest that the laterality hypothesis of major psychopathology may be especially relevant to the domain of memory functioning.

254.12 SEXUAL DIMORPHISM IN THE HUMAN CORPUS CALLOSUM? S. Demeter, J. Ringo, and R. W. Doty. Center for Brain Research, University of Rochester School of Medicine, Rochester, NY 14642.

The roles of "nature" and "nurture" in the determination of sex-linked differences in cognitive function have been much debated. The observation of anatomic variations in the cerebral cortical areas of men and women would bolster the arguments favoring a biological explanation. Since the corpus callosum consists largely of nerve fibers, which connect cortical areas of the two hemispheres, its morphology should reflect the presence or absence of such connections and thus serve as an indicator of this aspect of cortical structure. In a preliminary study, de Lacoste-Utamsing and Holloway (Science 216:1431-2, 1981) observed sexual dimorphism in the human corpus callosum, thus inferring sex-linked variation in cortical structure.

We have studied the corpora callosa of 19 men and 12 women obtained at autopsy and fixed in buffered formalin. The brains were weighed prior to fixation and were subsequently found to be free of significant neuropathology. Following fixation, each brain was bisected and photographed and its corpus callosum traced at a three-fold magnification. Computer-assisted planimetric measurements were made of the maximal splenial width, total callosal cross sectional area, and that of its posterior fifth, as described in the reference cited.

The maximal splenial width was observed to be the same in men and women and the cross-sectional area of the posterior fifth of the corpus callosum slightly larger in women than in men. However, this difference was not statistically significant. Moreover, three "blind" observers, provided with verbal descriptions and examples of tracings from the reference cited, failed to discriminate qualitatively between the corpora callosa of men and women at a better than chance level.

Although these observations suggest that there may, indeed, be a trend toward greater variability in the morphology of the corpus callosum between the sexes than within members of each sex, considerable caution must be exercised in attributing sex to a given brain on a morphological basis alone.

254.13 CEREBRAL ASYMMETRIES AND SEX DIFFERENCES IN HOMINOIDS. Darlene S. Horvath*, D.J. Woodward & Marie-Christine de Lacoste (SPON: D.L.O'Donoghue). Dept. Cell Biology, Univ.Texas Health Science Center, Dallas Texas, 75235

This study, which was undertaken to delineate the patterns of cortical asymmetries and sex differences in hominoids, is part of an overall research project designed (by MC de L) to investigate the phylogenetic and ontogenetic development of prosencephalic asymmetries in infrahuman anthropoid and prosimian primates, as well as in adult and fetal humans. Every 40th section (20 u in thickness) from coronal series of cerebra of Hylobates lar, Pan troglodytes and Gorilla gorilla, was photographed at the Max-Planck Inst. fur Hirnforschung (Frankfurt; Dr. H. Stephan's collection). The sections were digitized and five anatomical regions were delineated using allo- and neocortical landmarks, including the corpus callosum, hippocampus, amygdala, diencephalic nuclei and the parieto-occipital/calcarine sulci intersection, as landmarks. Regional volumes were obtained using the CARP system (fostered by DJW), SPSSx, and data transformation programs (written by M.C.deL) to interface between CARP and SPSSx.

Preliminary results suggest that patterns of asymmetry vary considerably among the hominoids. In the chimpanzee, all of the regions exclusive of the parietal association cortex (which was larger on the right side), manifested asymmetries favoring the left side. In the gorilla, the data suggest an alternate pattern of assymmetries with prerolandic cortex favoring the right side, and, conversely postrolandic cortex biased towards the left hemisphere. However, both of the pongid patterns of asymmetry in left/right regional volumes are different from the hominid pattern, although gorilla manifests closest approximation (similar results were obtained in an earlier study by Holloway and deLacoste, 1982). Furthermore, although Gorilla gorilla is one of the most sexually dimorphic pongids in terms of body and brain size, an initial analysis of the data indicates that there are only minor sex differences in gorilla prosencephalic asymmetries. While these results are only tentative, since the male brain used in this study was that of a juvenile gorilla, they are tantalizing because close relatives of the gorilla, Homo sapiens, exhibit significant sex diffferences in regional right/left hemispheric volumes.

In brief, our preliminary results suggest that in the course of evolution there may have been selective pressures on the hominid line for sex differences in cerebral asymmetry.

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254.14 RIGHT/LEFT PROSENCEPHALIC ASYMMETRIES IN LEMURIDAE. M.C.de Lacoste & D.J.Woodward, Cell Bio. UTHSC, Dallas, TX 75235

Ongoing research has demonstrated that hominoid brains manifest significant regional volumetric asymmetries. In this study we computed right/left regional volumes in various Lemuridae to determine if prosimians also exhibit prosencephalic asymmmetries. Every 20th coronal section (10-20 u in thickness) of cerebra from Microcebus murinus (N=6), Cheirogaleus major (N=2), Cheirogaleus medius (N=2), Lemur albifrons fulvus (N=2), Lemur mongoz (N=1) and Lepilemur ruficaudatus (N=3) was photographed at the Max-Planck Inst. fur Hirnforschung (Frankfurt). The body and brain weights of these species respectively range from 54 to 1,400g and 1.64 to 23.3g. The sections were digitized and prosencephalic regions were demarcated on the basis of species-specific anatomical landmarks. Regional volumes were obtained by using the CARP system (fostered by DJW), SPSSX, and data transformation programs (written by M.C. de L) to interface between CARP and SPSSX.

Preliminary results have been obtained on Microcebus murinus, a noctural lemur of Madagascar. Microcebus has the most primitive brain amongst extant lemurs, with a more developed olfactory bulb and a less conspicuous frontal association cortex than that found in other lemuroid genera; but, nonetheless with a triradiate calcarine complex, deep Sylvian fissure, distinct temporal pole and parietal association cortex. Landmarks used to demarcate regions in this species included the rhinal sulcus of the archicortex, the genu of the corpus callosum, and the only two sulci of the neopallium -- the Sylvian fissure and the calcarine complex.

Accordingly, the data indicate that although visually the Microcebus brain appears far more symmetrical than that of its hominoid relatives, only the regions encompassing primary motor, somatic sensory and auditory cortex are quantitatively symmetrical (diff R/L=.09%). Data on secondary somatic sensory areas were not uniform. However, the region approximating Brodmann's areas 6 and 8 was consitently asymmetrical, albeit not always in the same direction. Furthermore, striate and peristriate areas, as defined by the retrocalcarine sulcus, exhibited pronounced asymmetry (in some cases up to 13%). In addition, it was found that the Sylvian fissure was higher on the R-side in 75% of the cases, a finding which has not yet been described in non-anthropoid primates.

In sum, evidence of hemispheric differences in prosimians with sparse association cortex suggest that anatomical asymmetries play a role early in primate brain evolution. Supported by NSF BNS 8316764 (MC de L) & Biological Humanics (DJW).

254.15 COMPLEMENTARY HEMISPHERIC SUPERIORITIES IN MONKEYS.
C. R. Hamilton and B. A. Vermeire.
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We have shown previously that rhesus monkeys have 1) a left hemispheric (LH) superiority for learning to discriminate lines of different slopes (Behav. Bran Res. 1983, **10**, 399-403) and 2) a right hemispheric (RH) superiority for learning, remembering, or generalizing discriminations of facial features of monkeys (Soc. Neurosci. Abstr. 1983, **9**, 651). We have now confirmed and extended these findings with additional subjects and tasks, and have shown that differential superiorities for spatial and facial processing exist within the same monkey.

Spatial Discriminations. A significant ($p > 0.01$) LH advantage was found for 19 split-brain monkeys when each hemisphere was taught four discriminations between pairs of lines tilted by 15°. A LH advantage was also found for learning four other visuospatial discriminations although only one of these was significant. When the results from these five visuospatial tasks were pooled for each subject the LH superiority was highly significant ($p < 0.001$). The direction of this asymmetry is opposite to that usually reported for human subjects.

Facial Discriminations. A significant RH superiority was found for 25 split-brain monkeys that differentiated photographs of monkeys' faces in eight different discriminations. The hemispheric asymmetry was evident in learning ($p < 0.02$), remembering ($p < 0.01$), and generalizing ($p < 0.02$) the discriminations. The asymmetry was largest on the measure of retention, which was made about five months after the original learning. Overall, female monkeys showed greater hemispheric differences than males. The direction of the hemispheric asymmetry is the same as that usually reported for human subjects.

Complementary Specialization. Twelve of the subjects learned both the spatial and facial discriminations which allowed a direct comparison of hemispheric differences within each of these subjects. Eight of these monkeys showed a clear-cut reversal of hemispheric dominance for the two types of tasks. Eleven of the 12 showed relative differences in the expected direction for the two tasks (spatial dominance-facial dominance); the mean of these differences was highly significant ($p < 0.001$).

These results indicate that human-like hemispheric superiorities evolved before the development of language. The popular description of hemispheric specialization in human beings as "analytic vs holistic" may not be directly applicable to monkeys because the two "holistic" tasks we tested appear to be lateralized to opposite sides of the brain.

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## NEURAL BASIS OF BEHAVIOR: INTERHEMISPHERIC RELATIONS II

255.1 HISTOLOGICAL PARAMETERS IN CEREBRAL ARCHITECTONIC ASYMMETRY IN THE RAT. G.D. Rosen, F. Aboitiz*, G.F. Sherman, and A.M. Galaburda. Neurological Unit, Beth Israel Hospital and Harvard Med. School, Boston, MA 02215.

In the rat, the primary visual cortex (area 17) has been shown to exhibit absolute volume asymmetries (Sherman and Galaburda, Soc. Neurosci. Abs., 8:627, 1982). These asymmetries may be the result of side differences in cell-packing density (governed by cell size and interneuronal elements), or in total cell numbers, or both. The present experiment was designed to distinguish among these possibilities.

Nineteen (9 male and 10 female) adult Purdue-Wistar rats were perfused transcardially with formalin and their brains embedded in celloidin, sectioned coronally at 30μ, and every tenth section stained for Nissl substance. Parcellation of the entire neocortex and of area 17 of both hemispheres was performed under light microscopy. The parcellations were projected with a distortion-free projection apparatus, traced, and the volumes determined using an electronic planimeter. Cell-counts within area 17 were carried out at a 500X magnification using a rectangular reticule. For each animal, cell counts were performed on three slides (sampling from the rostral-caudal extent of the area). On each slide, neurons were counted in each of layers II-VI in two adjacent fields in both the right and left hemispheres. Thus, for each animal, 60 fields were counted. All measures were performed blind with respect to hemisphere and sex. The asymmetry coefficient (AC) was derived using the formula: $AC=(R-L)/0.5(R+L)$.

There were no sex differences, so the data were collapsed over this variable. There were significant absolute asymmetries in total cortical volume ($AC=0.016$, $t=5.83$, $df=18$, $p < 0.001$), in the volume of area 17 ($AC=0.074$, $t=5.39$, $df=18$, $p<0.001$), and in cell-packing density ($AC=0.043$, $t=9.05$, $df=18$, $p<0.001$) although there was no intercorrelation among these measures. Because there was no correlation between asymmetry in cell-packing density and area 17 volume asymmetry, it is concluded that interhemispheric differences in cell numbers, and not packing density, account for the asymmetry in the volume of area 17. Also, since total neocortical asymmetry does not predict for asymmetry in a given architectonic area such as 17, statements about structure/function relationships are not warranted from gross anatomical asymmetry data. In addition, a significant negative correlation was found between the total volume of area 17 (i.e., R+L) and the absolute volumetric asymmetry of that area ($r=-0.489$, $n=19$, $p<0.05$). Thus, brains with a smaller area 17 are more asymmetrical in that area.

We will discuss possible developmental mechanisms for anatomical asymmetry, as suggested by these results. (Supported by NIH grants 14018 and 19819).

255.2 EFFECTS OF INFANTILE HANDLING AND UNILATERAL CORTICAL LESIONS UPON SPATIAL PREFERENCE AND MAZE LEARNING IN THE RAT. A.S. Berrebi, D.A. Yutzey, S.H. Freter*, T.F. Pianta*, and V.H. Denenberg. Depts. of Biobehavioral Sciences & Psychology, Univ. of Conn., Storrs, CT 06268.

Previous research using unilateral neocortical lesions found that different behavioral responses can be obtained from rats with left (L) or right (R) cortical lesions, suggesting an underlying asymmetry in cerebral organization. Denenberg and his colleagues have also shown that extra stimulation in infancy (handling) can serve to modify the behavioral organization of the rat, often resulting in the enhancement of behavioral asymmetry (Beh. Br. Sci., 4: 1981). Additionally, Sherman et al. (Br. Res., 192: 1980) found that a left lesion (L) had greater influence upon directional preference than did a right lesion (R) in standard laboratory rats. This experiment examined the hemispheric laterality of the female Purdue-Wistar rat in a water maze of the Lashley III type.

Rats were handled or not handled during the first 20 days of life. When adult, all animals were given a unilateral decortication (L or R) or a sham lesion. They were given ample recovery time before testing began. A scaled drawing of the Lashley III maze was used to record the path taken by each animal, thereby allowing for the assessment of total, initial, and directional errors made in each of 19 trials. We only report initial and directional errors here.

While all lesioned animals made more errors in the direction ipsilateral to their lesions, L animals did so significantly more than R animals, indicating an underlying cerebral asymmetry and implicating the right hemisphere as the primary mediator of spatial preference in the rat. Furthermore, the difference in the strength of the spatial bias between L and R animals was greater in the handled animals than in the nonhandled animals throughout the test sessions.

Analyses of the learning curves found that rats with an intact right hemisphere had smoother and less variable learning curves than those with an intact left hemisphere. Also, those with the right hemisphere intact had sharper separation between their right and left directional errors than did those with an intact left hemisphere. Finally, infantile handling experience was found to interact with site of lesion.

255.3 HEMISPHERIC ASYMMETRY AND INTERACTION FOR RAT PAW USE. F. Szeligo, N. Clarke, E. Stillwell, N. deVries, and H. Wishart. Psyc. Dept., Univ. of New Brunswick, Fredericton, N.B., Canada E3B 6E4

Individual, non-human animals demonstrate strong preferences for left or right, but not the type of cross-population preferences seen for humans. The explanation seemed to be the specialization and lateralization of the human brain. However, evidence accumulates for anatomical, biochemical and functional dichotomization of animal hemispheres, without corresponding demonstration of behavioural lateralization.

The population of rats in the present study depicts the usual situation. In an unselected series, there were equivalent numbers of rats with left and right paw preferences (lp and rp, respectively). Further, the majority of these rats never made a 'normal' reach with the non-preferred paw (npp) during the period of testing reported. Given the strength of preference and the presumed presence of neural asymmetry, the equivalence of group preferences seems paradoxical. An entry into the resolution of this paradox, however, seems to be present.

Food deprived rats reached into narrow slots for pellets (Castro, A.J. Brain Res. 37: 173-185, 1972) during 10, 10 second trials/day for 10 days. Then some received damage to the anterior callosum and an additional 5 days of training.

On a 'normal' reach the rat's snout enters a slot and the preferred paw (pp) comes into the same slot; if the rat is to use its npp, the pp will retract and they will alternate reaching into the slot. Based on this type of reach there were 28 rp rats and 29 lp rats. On relatively rare occasions (although in some rats it approaches 1:1), the npp will reach simultaneously with the pp and enter a slot next to the slot occupied by the pp and head (-h). The rat, having asymmetrically oriented for a pp reach, has simultaneously maneuvered the npp into a position that leads to an off-target reach. The -h reach is usually without reward. This -h reaching was present in 15/28 rp and 6/29 lp rats. The proportions are significantly different ($Z = 2.54$, $p < .02$). Following damage to the callosum 2/15 rp rats increased the amount of -h reaching (only 3 produced any); 8/16 lp rats increased the amount of -h reaching ($Z = 2.18$, $p < .03$). More dramatically, 1/16 rp rats went from 0 to some -h; 6/14 lp rats went from 0 to some -h.

The pattern of results suggests that the consequences of neural asymmetry are evident, but only in ancillary behaviour. The pattern further suggests that the source of -h reading is the left hemisphere. The presence of -h reaching seems to represent a lower threshold in the left hemisphere for the production of behaviour such that it is produced even when conditions of environment and rat are not appropriate.

255.4 DIFFERENTIAL DISTRIBUTION OF H-2 HAPLOTYPES IN LINES OF MICE SELECTIVELY BRED FOR DEGREE OF LATERALIZATION. R. L. Collins, G. A. Carlson* and J. H. Nadeau*. Jackson Laboratory, Bar Harbor, ME 04609.

Eleven generations of bidirectional selective breeding produced two lines of mice differing markedly in degree of lateralization for hand preference (Collins, in S. D. Glick (Ed.), Cerebral Lateralization in Nonhuman Species, Academic Press, 41-70, 1985). Geschwind reported that immune disorders were clustered in left-handers, proposed this association was mediated by fetal androgen, and suggested that MHC genes controlling immune responsiveness also regulate the testosterone effect (Geschwind and Behan, PNAS 79: 5097-5100, 1982). The H-2 complex in mouse is the MHC homolog of HLA in man. If differences of lateralization are influenced by genes within or near the H-2 complex, we should expect that H-2 haplotypes will be differentially distributed between HI and LO lines of mice. Preliminary evidence confirms this pattern.

The foundation population was derived from 8 progenitor stocks: C57BL/6J, BALB/cJ, DBA/2J, LP/J, SM/J, RF/J, M. molossinus and M. castaneus. Of these, 6 differ in H-2 haplotype. Selection was stopped at G12, and lines were maintained by random mating since. Parental pairs of G24 HI and LO lines were typed for H-2 using a modified Terasaki microcytotoxicity procedure. H-2 haplotypes were distributed as follows:

| | b | d | k | wc | wm | v |
|---|---|---|---|---|---|---|
| GO | .285 | .240 | .128 | .128 | .086 | .131 |
| HI | 0 | .471 | 0 | .132 | .25 | .147 |
| LO | .546 | .030 | 0 | .01 | .172 | .242 |

Haplotypes b and d are differentially distributed in HI and LO lines, and k is absent from both. This pattern could arise by pleiotropy, by genetic linkage of target and H-2 loci, or by chance. The elimination of neutral genes by stochastic losses in finite populations with effective population size Ne = 48 would take longer on average than that observed; for b, 96 generations; for d, 86; for k, 58 (p. 431 of Crow and Kimura, An Introduction to Population Genetic Theory, Harper & Row, New York, 1970). The distribution of genetic variants of loci located elsewhere were obtained in the same mice: Aco-1 (Chr 4), Idh-1(1), Es-3(11), Gpi-1(7), Pep-3(1), Pgm-1(5), Es-10(14), and Hbb(7). Allelic frequencies for these loci did not exhibit the mirror-image pattern shown by H-2 haplotypes. Thus, the differential distribution of H-2 haplotypes between selected lines appears to be nonrandom. This suggests either that the H-2 complex itself exerts a pleiotropic influence on degree of lateralization, or that genes affecting lateralization reside at loci located near the H-2 complex on Chromosome 17. (Supported by GM 23618, GM 32461, CA 28231.)

255.5 STRESS-INDUCED CHANGES IN ROTATIONAL BEHAVIOR. J. Carlson* and S.D. Glick. Dept. of Pharmacology and Toxicology, Albany Medical College, Albany, N.Y. 12208

Previously this laboratory observed differences in cocaine-induced rotational behavior between left and right biased rats (S.D. Glick et al., Science, 221:775, 1983). Right-rotating female rats rotated much more than did left rotators while left rotating males rotated much more than right rotators. No sex or bias dependent differences were seen on rotation in response to d-amphetamine. In light of previous data suggesting that stress may modify an organism's reaction to stimulant drugs (A.J. MacLennan et al., Science, 219:1091, 1983) and other data (A.M. Thierry et al., Nature, 263:242:1976; N.E. Goeders and J.E. Smith, Science, 263:773, 1983) suggesting that stress and cocaine may influence similar neuronal systems (i.e., mesocortical dopamine), experiments were undertaken to examine alterations in rotational behavior to d-amphetamine in rats exposed to controllable or uncontrollable stress. Following an assessment of d-amphetamine induced rotational behavior (1.56 mg/kg males; 1.25 mg/kg females), male and female rats were exposed six days later to a series of 80 1 mA footshocks which were escapable with an appropriate response (controllable stress) or identical in duration and inescapable (uncontrollable stress). On the following day, each rat's rotational behavior in response to d-amphetamine was again tested as done previously. Analysis of rotational behavior, as indicated by directional preference before and after stress, revealed that, when compared to non-stressed controls, the rotational behavior of stressed animals was altered in a manner that was dependent upon pre-stress directional bias. Right rotating males showed a stress-dependent reduction in right preference with left rotating males being unaffected, and left rotating females showed a similar reduction with right rotators being unaffected. In addition, differences were noted in animals exposed to uncontrollable as opposed to controllable stress, suggesting that the opportunity to control the stress is an important variable in effecting these changes. These findings suggest that stress and cocaine share some commonality in producing lateralized changes in the CNS, that the ability to cope with stress is important in determining the magnitude of this change, and that individual differences in reactivity to stress may be determined in part by lateralized differences in CNS function (Supported by NIDA grant DA 03817).

255.6 RIGHT>LEFT ASYMMETRY IN D-2 BINDING IN FEMALE RAT STRIATUM K.L. Drew*, R.A. Lyon*, M. Titeler, and S.D. Glick. (SPON: F.L. Rice) Department of Pharmacology and Toxicology, Albany Medical College, Albany, New York 12208

Normal rats are known to rotate (turn in circles) in response to drugs (e.g., d-amphetamine) during the day and without drugs at night. Asymmetries in striatal dopamine and dopamine metabolites have been related to the directions of female rats' turning preferences. Schneider et al.(1) reported 23% more $^3$H-spiroperidol (D-2) binding in the left than in the right striatum in male rats. No behavioral data were available, however, so it was unclear if the left-right difference was, or was not, related to any functional asymmetry. The present study was conducted to determine if a left-right asymmetry in D-2 binding also occurs in female rats, and if such an asymmetry is related to individual rotational preferences.

Female rats (N=22) were tested in rotometers at night during the active portion of their diurnal cycle. Left and right striata were dissected, frozen on dry ice and stored at -70$^{o}$C. In order to quantitate the receptor densities (Bmax), independent Scatchard analyses were performed on each striatum using $^3$H-N-Methyl Spiperone ($^3$H-NMSP) for total binding and sulpiride to define non-specific binding. Six concentrations of $^3$H-NMSP were used ranging from $10^{-11}$ M to $10^{-9}$M. The final tissue concentration was 0.75 mg/ml and protein determinations were done by the method of Lowry. Kd and Bmax values were calculated using the radioligand binding program EBDA (2).

D-2 receptor densities were significantly greater in the right striatum than in the left striatum while no difference in Kd values was found. There was no relationship between rotational direction and Bmax asymmetry.

| | R | L | |
|---|---|---|---|
| Bmax(fM/mg) | 180 | 140 | p<.02 (paired t-test) |
| Kd($\times 10^{-11}$) | 11 | 10 | N.S. |

The asymmetry reported here for female rats is greater in magnitude and opposite in direction to that previously reported for male rats(1). Although the functional significance of this D-2 asymmetry is as yet unknown, such an asymmetry needs to be considered when evaluating changes after drugs or lesions. (supported by NIDA grant DA 03817 and BRSG grant SO7RR05394-23).

1. Schneider L.H. et al., Neurosci Lett, 33: 281, 1982
2. McPherson G.A., Comput Programs Biomed, 17: 107, 1983

255.7 Differential effect of cortical versus subcortical noradrenergic lesions on lateralized spontaneous hyperactivity. Kenneth M. Saad[1,2]*, Catherine Mikat*, Timothy H. Moran[1], Robert G. Robinson[1,2]. [1]Dept. of Psychiatry and Behavioral Sciences, and the [2]Dept. of Neuroscience, The Johns Hopkins University School of Medicine.

Previous studies have demonstrated that noradrenergic lesions produced by as little as 1 ug of 6-hydroxydopamine injected into the right frontal lateral cortex of the rat will produce spontaneous hyperactivity while symmetrical lesions of the left hemisphere will not (Robinson & Stitt, Brain Res. 213:387, 1981). The present study assessed whether unilateral lesions of the subcortical noradrenergic dorsal bundle would also produce lateralized hyperactivity.

Male rats were given unilateral lesions of the noradrenergic dorsal bundle at 6.2 mm posterior to bregma 1.1 mm lateral to the midline and 6.2 mm from surface using either electrolytic (1 ma for 3.5 sec.) or 6-hydroxydopamine (6-OHDA) (2 or 4 ug in 1 ul solution injected at 0.33 ul/min). Seven days preoperatively and 30 day intervals by postoperatively spontaneous activity was measured at 24 hour running wheel revolutions or alternatively at once per week intervals over the 12 hour dark cycle open field activity was measured (total distance travelled) in computerized chambers with infrared detectors (Omnitech Electronics, Columbus, Ohio). There were no significant differences in spontaneous activity using photocell chambers or running wheels between right or left lesion animals with either electrolytic or 6-hydroxydopamine lesions. The lesion animals, however, were significantly more active than controls ($F_{1,23} = 4.76$ $p < .05$ electrolytic $F_{1,23} =$ , $p < .05$ 4 ug 6-OHDA). Biochemical measurement of norepinephrine concentrations following electrolytic lesions demonstrated significant ipsilateral depletions of norepinephrine in the frontal and posterior cortex ($F_{2,42} = 5.9$ $p < .01$ frontal; $F_{2,42} = 5.4$ $p < .01$ posterior). There was a significant increase in ipsilateral vs.contralateral NE concentrations in the locus coeruleus ($F_{2,42} = 4.5$ $p < .05$). The biochemical results of the 6-OHDA lesions are in process and will be presented at the meeting. These findings indicate that unilateral noradrenergic lesions of the dorsal bundle produce symmetrical effects on spontaneous activity. This is in contrast to the effect of cortical noradrenergic lesions. Thus, subcortical noradrenergic lesions may have different physiological effects on the cortex than cortical noradrenergic lesions. These data might also suggest that left hemisphere cortical injury may produce an inhibitory effect on activity.

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255.8 INTERHEMISPHERIC COORDINATION OF BIRD SONG. H. Williams* (SPON: J.A. Paton). Rockefeller University, Millbrook, NY 12545.

In song birds, vocalization is controlled by a chain of telencephalic nuclei, NIf → HVc → RA; RA projects to the motor neurons innervating the vocal organ, the syrinx, which provides two independent sound sources (Nottebohm et al., JCN, 207:344, 1982). Each half of the syrinx is innervated by the ipsilateral NXIIts (tracheosyringeal nerve). Cutting one nerve or lesioning one HVc grossly distorts certain syllables or notes while leaving the remainder of the song intact (177:229, 1971; Nottebohm et al., JCN, 165:457, 1976). Single-pulse microstimulation of either HVc elicits a volley only in the ipsilateral NXIIts (Paton and Manogue, JCN, 212:329, 1982). These results strongly suggest that each hemisphere independently controls a certain portion of the bird's song (Nottebohm & Nottebohm, J. Comp. Physiol., 108:171, 1976). Yet the notes and syllables which make up bird song are delivered with exquisitely coordinated timing.

One possible mechanism for interhemispheric coordination would be contralateral projections to the forebrain song nuclei, perhaps via the anterior commissure (birds do not have a corpus callosum). However, HRP injections into RA, HVc, and NIf failed to backfill any neurons in the contralateral half of the brain.

The two known non-auditory inputs to NIf, HVc and RA are MAN and uva. Microstimulation of MAN, a magnocellular nucleus of the anterior forebrain, evokes responses only in the ipsilateral HVc and RA. Therefore, MAN cannot play a role in interhemispheric communication. Uva is a small thalamic nucleus projecting to the ipsilateral NIf and HVc and the contralateral uva, and receiving inputs from laryngeal motor neurons (Nottebohm et al., JCN, 207: 344, 1982). Microstimulation of uva elicits firing in the ipsilateral HVc, followed by a volley in the ipsilateral NXIIts. The conduction times are appropriate for an uva → NIf → HVc → RA → NXIIts pathway. Stimulating uva also evokes a response in the contralateral HVc and NXIIts, with latencies 3-5 ms longer than those recorded ipsilaterally. The difference in latency suggests that firing in uva excites the cells in the contralateral uva, in turn exciting cells in the nuclei of the contralateral song system. This hypothesis was confirmed by the finding that, after the contralateral uva was lesioned, uva stimulation elicited responses only in the ipsilateral HVc and NXIIts.

Thus, uva seems to provide the only direct non-auditory bilateral input to the song system. If the projection to uva from the laryngeal motor neurons carries information on the state of the air passage in the larynx, the inputs to HVc and NIf from the linked uvas would allow the song control nuclei in each hemisphere to synchronize their activity with the pattern of airflow in the vocal tract - thus coordinating the patterns of syringeal muscle contraction and breath control to yield complex but ordered song.

255.9 INTERHEMISPHERIC TRANSFER OF VISUAL DISCRIMINATION IN PIGEONS WITH UNILATERAL THALAMIC LESION. T.Shimizu,S. Watanabe,W.Hodos and B.Bessette. Dept. of Psychology, Univ. Maryland,College Park,MD 20742.

Interhemispheric transfer of monocularly learned discrimination was studied in pigeons with unilateral thalamic lesion. The pigeon has two visual systems,i.e., the thalamo-fugal pathway and the tecto-fugal pathway. At the level of thalamus,OPT is a relay in the former pathway and nucleus rotundus is a relay in the latter pathway. Selective lesion of these two structures was made after monocular learning and interocular transfer of the learning was examined.

METHOD

The experimental chamber was an operant chamber with two pecking keys on which discriminative stimuli were displayed by a carousel projector. Two groups of pigeons were monocularly trained on intensity,color and pattern discriminations. Following completion of discrimination training,unilateral electrolytic lesion of OPT or nucleus rotundus was made stereotactically. One group received the lesion in the trained hemisphere(contralateral to the trained eye),and the other group received the lesion in the untrained hemisphere. After 7 days of postoperative recovery period,both groups were retrained on the same task with the previously untrained eye to examine the interhemispheric transfer of learning. After behavioral experiment,the brains of the birds were histologically examined under the microscope to reconstruct the lesions. The birds without any damage in the visual system were used as control animal.

RESULTS

The OPT lesion,in either hemisphere,had no effect on interhemsipheric transfer,whereas,the lesion of the nucleus rotundus in the trained hemisphere produced a deficit in transfer. The rotundal lesion in the untrained hemisphere also yielded a deficit in transfer.

CONCLUSION

These results suggest 1) the tecto-fugal pathway has a cruicial role in interhemispheic transfer of visual discrimination in pigeons,and 2) formation of unilateral memory storage by monocular learning.

256.1 ADRENERGIC ANTAGONISTS: ATTENUATION OF MEMORY FACILITATION. D.B. Sternberg and J.L. McGaugh. Center for the Neurobiology of Learning and Memory and the Department of Psychobiology, University of California, Irvine, CA 92717.

Previous research indicates that, retention of many types of training tasks is enhanced by post-training administration of epinephrine and that the memory-enhancing effect of epinephrine is blocked by propranolol, a β-adrenergic antagonist, administered prior to training. The present study examined the effect of several α- and β-adrenergic antagonists administered prior to training to determine whether the effects of post-training administration are selectively attenuated by β-adrenergic antagonists.

Male Charles River Sprague-Dawley rats (60-90 days old) were trained in a one-trial inhibitory (passive) avoidance task. Animals were water-deprived and maintained at 85-90% of their original weights. Animals received a total of five pre-training sessions over a three day period. Animals were pre-trained to go to the dark end of the training apparatus to drink water. On the training day, thirty minutes prior to training, animals received an i.p. injection of either an α1-adrenergic antagonist, prazosin, α2-adrenergic antagonist, yohimbine, α1- and α2-adrenergic antagonist, phentolamine, a β1-adrenergic antagonist, atenolol, β2-adrenergic antagonist, zinterol, β1- and β2-adrenergic antagonist, propranolol, or the α1- and α2, β1- and β2-adrenergic antagonist medraxolol or saline. During training, the animals received relatively low footshock (350 μA, 0.7 sec) after drinking for 10 secs. Immediately following training the animals received a s.c. injection of epinephrine or saline. Twenty-four hours later, the animals were tested for retention by measuring their latency to lick. The retention performance of animals given pre-training saline injections and post-training epinephrine injections was significantly higher than that of saline control animals. Animals which received pre-training injections of any of the adrenergic blocking agents and post-training epinephrine or saline had retention scores comparable to those of the saline controls.

These findings indicate that the retention enhancing effects of post-training epinephrine are blocked by a wide variety of α- and β-antagonists.

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256.2 THE INVOLVEMENT OF CALCIUM, GLUTAMATE, AND PROTEIN SYNTHESIS IN SHORT- AND LONG-TERM WORKING MEMORIES OF THE RAT. S. J. Y. Mizumori, V. Channon*, M. R. Rosenzweig, and E. L. Bennett. Department of Psychology, Univ. of Calif., Berkeley CA 94720.

Previous experiments suggest working memory of rats trained in a radial maze can be discussed in terms of short- and long-term temporal components. Long-term working memory (LTWM) was found to be susceptible to disruption by the protein synthesis inhibitor, anisomycin (Mizumori et al., Behav. Neurosci., 99: 220, 1985). Experiment 1 of this study examined the neuropharmacological nature of the short-term component to working memory (STWM). Experiment 2 addressed the issue of whether LTWM in a radial maze involves protein synthesis either because items are to be remembered over a relatively long period of time, or because the radial maze is a relatively complex task requiring storage of large amounts of information.

For Experiment 1, rats were trained to criterion on a 12-arm radial maze with 240-min delays imposed between choices 6 and 7. On test days, one of the following drugs was injected into ventral hippocampus (via chronic indwelling cannulae) 30 min before choice 1: saline (SAL), 40 mM $LaCl_3$ (a calcium uptake inhibitor), 1 M glutamate (GLU), or 302 mM anisomycin (ANI). Rats were tested after 0-, 2-, 8-, or 15-min delays. Both $LaCl_3$ and GLU injections resulted im impaired choice accuracy, regardless of the length of the delay interval ($p < .05$). ANI injection did not affect performance when 0- or 2-min delays were used; significant impairment was found following 8- or 15-min delays. Together, these data support the hypothesis that working memory involves at least two temporal components--the short-term component may involve changes in calcium uptake and the glutaminergic system, while the long-term component involves protein synthesis.

If ANI impaired LTWM in the radial maze because proteins were important for holding information over relatively long periods of time, then ANI should also impair LTWM in a simpler 2-choice spatial task--delayed alternation (DA). On the other hand, if protein synthesis was necessary because the radial maze is a complex task, then ANI might not impair choice accuracy after long delays for rats trained in the DA task. Experiment 2 showed that ANI impaired working memory in a DA task, but only when delay intervals of 8-min or longer were used. ANI had no effect on memory following 2-min delays. Thus, the temporal course of development of ANI-induced amnesia was the same for both radial maze and DA tasks. This suggests protein synthesis is important for working memory when items are to be remembered for a relatively long period of time, and may be less related to the number of items to be remembered.

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256.3 THE INVOLVEMENT OF CALCIUM, GLUTAMATE, AND PROTEIN SYNTHESIS IN AVOIDANCE LEARNING IN MICE. D. H. Sakai*, S. J. Y. Mizumori, M. R. Rosenzweig, and E. L. Bennett. Department of Psychology, Univ. of Calif., Berkeley, CA 94720.

Results of different approaches to the study of the chemical basis of learning (e.g., invertebrate models, long-term synaptic enhancement [LTE], and avian conditioning) suggest that behavioral learning may involve changes in calcium regulation and the glutaminergic system. However, systematic analysis of the role of these chemical systems in mammalian behavioral learning has not been done. Here we tested the effects of agonists and antagonists to the calcium and glutamate systems on mouse avoidance learning. Furthermore, attempts were made to prevent amnesia by simultaneous injection of calcium or glutamate agonists and antagonists. Finally, an assessment of the behavioral effects of anisomycin (ANI--a protein synthesis inhibitor) was included for comparison, since based on earlier reports, ANI is known to impair memory for avoidance learning in mammals.

One of the following agents was injected into dorsal hippocampus of mice 45 min before one-way active avoidance training: saline (SAL), $LaCl_3$ (a calcium uptake inhibitor), γ D-glutamylglycine (γDGG--a glutamate receptor antagonist), $CaCl_2$, glutamate (GLU), ANI, or a combination of $LaCl_3$ + $CaCl_2$ or GLU ∓ γDGG. Retention tests were conducted 2 days after training.

Both $LaCl_3$ (40 mM) and GLU (1 M) produced significant amnesia. Test scores of mice injected with $CaCl_2$ (20, 40, or 60 mM) or γDGG (0.1 or 0.5 mM) did not differ from SAL-injected controls. 40 mM $CaCl_2$ prevented the amnesic effect of $LaCl_3$, but 20 or 60 mM $CaCl_2$ did not. When γDGG and GLU were injected simultaneously, test scores were intermediate between those of SAL- and GLU-treated mice. It appears that γDGG, in the doses employed, only partially prevented GLU-induced amnesia. ANI (604 mM) treatment rendered mice amnesic at test.

The behavioral effects of $LaCl_3$, ANI, and $CaCl_2$ were in accord with conclusions drawn from experiments on Aplysia, LTE or avian conditioning. Results of GLU or γDGG injections support the findings of avian conditioning experiments but not LTE experiments. Possible interpretations of this discrepancy will be discussed.

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256.4 GLYCOGENOLYSIS AS A MODULATOR OF MEMORY STORAGE. J.L. Hall*and P.E. Gold. Dept. Psychology, University of Virginia, Charlottesville, VA 22901.

Peripheral epinephrine (EPI), released from the adrenal medulla, is a major component of those systems which regulate the storage of new information. Posttraining injections of EPI enhance later retention performance in a variety of tasks and circulating EPI levels predict later retention performance. Furthermore, the effects of many treatments on memory are blocked in animals pretreated with peripherally administered adrenergic antagonists. The present studies evaluate the possibility that EPI effects on memory may be the result of the hormone's action on the liver to induce glycogenolysis.

Rats were trained in a one-trial passive (inhibitory) avoidance task using a relatively mild footshock (0.7 ma, 0.5 sec). Immediately after training, the animals received an injection of glucose (GLU; 0.01-10 mg/kg), EPI (0.1 mg/kg) or saline. On the test trial 24 hr later, animals which received EPI or GLU (0.1 and 1 mg/kg) had significantly higher retention scores. Injections delayed by 1 hr after training had no effect on later performance. Thus, GLU injections retroactively enhanced memory storage. In contrast to the results obtained with EPI and other memory-impairing and -enhancing treatments, pretraining injections of adrenergic antagonists, did not attenuate the effects of GLU on memory. Thus, glucose appears to represent a step in memory modulation subsequent to EPI release.

Further studies used animals with chronic tail artery catheters from which plasma samples could be taken at several times after training and drug treatment. The plasma samples were assayed for GLU using a colorimetric hexokinase procedure. The results indicated that plasma GLU levels were increased significantly above basal values for up to 30 min after footshock training. Injections of GLU or EPI at doses which enhance memory also resulted in increases in plasma GLU levels for at least 30 min.

These results suggest that the following series of events may be important in regulating memory storage for avoidance training. Footshock results in release of EPI, controlled by splanchnic afferents to the adrenal medulla and EPI, in turn, releases hepatic GLU stores which then modulate memory storage. Thus, GLU availability may regulate central neuronal changes by controlling the substrate necessary for intermediary metabolism in neurons. This conclusion is consistent with evidence that brain pyruvate dehydrogenase, an enzyme at a critical point in GLU utilization, is activated after training experiences comparable to those used here (e.g., Morgan and Routtenberg, Science, 1981, 214, 470-471).

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256.5 AVERSIVE EFFECTS OF LITHIUM MAY BE MEDIATED BY NEUROHYPOPHYSEAL HORMONES. E.F. O'Connor, S.W.T.Cheng* and W.G. North.* Dept. of Physiology, Dartmouth Medical School, Hanover, NH 03755

Vasopressin (AVP) appears to facilitate memory processing and learning either directly or as a consequence of its action in arousal. Several researchers have suggested that AVP's actions on behavior and physiology should be compared with those changes induced by Lithium salts (Li), which are known aversive agents and like AVP also stimulate learning and memory. In our study we examined changes in blood pressure (MAP) and in plasma levels of AVP and oxytocin associated neurophysins (NPs) in response to intra peritoneal injection of lithium chloride at 3.0 and 1.5 mEq/kg Bwt., doses which are used in behavioral studies and which approximate therapeutic levels. Control animals received .9% NaCl in an equivalent volume (.1ml/100g Bwt.). Blood pressure increases were induced by both doses of LiCl but not after saline. Both doses of LiCl also significantly increased plasma osmolality ($p<.05$), plasma Li($p<.001$), and plasma concentration of both AVP- and OT- associated neurophysins ($p<.001$).

Increases in AVP-NP were correlated with pOsm ($r=.75, p<.05$) and pLi ($r=.88, p<.001$). Comparisons of the two neurophysins suggests that in the first 10 minutes after injectin, plasma OT-NP levels increases rapidly and at 20 minutes begin to decline. In contrast, AVP-NP levels increase more slowly but continue to increase steadily for at least 60 minutes following bolus injection of LiCl. Since NPs provide an index of the function of AVP and OT neurons our results suggest that vasopressin and oxytocin are released in response to i.p. LiCl. Thus AVP and OT or their metabolites may be important in the mediatino of LiCl aversive effects or conversely, that the release of these hormones may directly mediate Li effects on learning.

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256.6 EFFECTS OF PRE- VS. POST-TRAINING INJECTION OF ANISOMYCIN ON MEMORY FOR A NOVEL ACTIVE AVOIDANCE TASK. S. Benloucif, M.R. Rosenzweig, E.L. Bennett. Dept. of Psychology, Univ. of California, Berkeley, CA 94720.

Experiments with the protein synthesis inhibitor anisomycin (ANI) in this novel active avoidance task indicate greater effectiveness of ANI when administered after training than prior to training. Other tasks have consistently shown a greater effect with pre-training injections. This work examines the effect of the injection procedure on ANI induced amnesia in a Wall Jump (WJ) task.

The WJ is a 10 X 32 X 21 cm plexiglas box with a dividing door and floor shock grid. The 3 escape compartment walls are lined with wire mesh. Charles River male CD1 mice (50 to 90 days) received 3 training and 10 test trials. Trials consisted of 10 sec adaptation in the start compartment, then simultaneous opening of the door and start of a buzzer, followed 5 sec later by mild footshock (.30 mA). A correct response consisted of avoiding the shock by climbing onto the wire mesh. Test trials were conducted at 1, 4, or 7 days. Mice were injected with 120 mg/kg ANI subcutaneously (SC) or 160 µg ANI into the hippocampus (IC) following Metofane anesthesia. Control mice were injected with equal volumes of saline (.01 ml/gm body wt SC or .5 µl per hemisphere IC). The mean number of avoidances at test was compared by analysis of variance. Results shown are significant at the .05 level or beyond.

Table of Results

| injection times pre | post | injection type | no. experiments showing impairment |
|---|---|---|---|
| single injection | | | |
| 15' | | SC | 1 of 3 |
| | 15' | SC | 3 of 3 |
| 45' | | IC | 0 of 1 |
| | immed | IC | 1 of 1 |
| double injection | | | |
| 15' ANI | 15' sal | SC | 0 of 1 |
| 15' sal | 15' ANI | SC | 0 of 1 |

A single post-training injection of ANI, either SC or IC, consistently impaired performance at test (4 of 4 expts.). A single pre-training injection impaired performance in only 1 of 4 expts. The series of double injections (to date incomplete) supports the hypothesis of an interaction of injection procedure and amnesic effect of ANI. Two possible explanations for the results are these: (1) Giving injections prior to training may impair acquisition in this task, obscuring an effect of ANI. (2) An injection given after training may increase the efficacy of ANI, causing amnesia for this task. Further work will test the two hypotheses.

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256.7 FAILURE OF NOOTROPICS TO MODULATE SEPTOHIPPOCAMPAL NEURONS IN THE RAT. M.F.Piercey and G.D.Vogelsang, CNS Research, The Upjohn Company, Kalamazoo, MI 49001.

High affinity choline uptake in the hippocampus has been reported to be stimulated by the nootropics piracetam (Sethy, 1983, Neurosci. Abs. 9:429) and pramiracetam (Pugsley, Poschel, Downs, Shih & Gluckman, 1983, Psychopharm. Bull. 19:721). This effect might be explained by an increase in firing rates of cholinergic neurons projecting to the hippocampus; higher firing rates would cause choline uptake to increase in response to the high demand for new synthesis of acetylcholine. Increases in firing rates of these cholinergic neurons could be responsible for the memory enhancement properties of nootropics since both acetylcholine and the hippocampus have been implicated as being important in memory function.

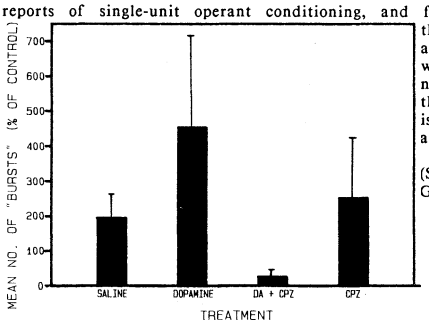
Using microelectrode recordings in urethane-anesthetized rats, we have now directly studied the effects of nootropics on firing rates of neurons projecting to the hippocampus from its major cholinergic input in the medial septum. Septohippocampal neurons were identified by constant short latency all-or-none responses to antidromic stimulation of the fimbria. Such cells followed the antidromic stimulus faithfully at high frequencies. Spontaneous impulses from these cells "collided" with evoked impulses, confirming that the cells were truly projecting to the hippocampus. A separate group of septal neurons was orthodromically (synaptically) excited by fimbrial stimulation. Histological recovery of dye spots left at the ends of the experiments revealed that antidromically invaded cells were located in the medial septum. Orthodromically activated cells were almost always in the lateral septum.

Piracetam and pramiracetam were tested for their effects on identified septohippocampal neurons. Although a wide variety of doses were tested and observations made over fairly long time periods, neither nootropic agent affected the firing rates of septohippocampal neurons.

The results suggest that the effects on choline uptake may be due to something other than increases in firing rates of cholinergic neurons.

256.8 OPERANT CONDITIONING OF HIPPOCAMPAL NEURONS: CHLORPROMAZINE BLOCKS REINFORCING ACTIONS OF DOPAMINE. *L. Stein and J. D. Belluzzi.* Department of Pharmacology, College of Medicine, University of California at Irvine, Irvine, CA 92717.

Although great efforts have been made to identify neurons that may deliver reward messages, the neurons or neuronal organizations that may receive such messages have been given little consideration. We have assumed that individual neurons may be organizational units whose behavior is capable of positive reinforcement. Operant conditioning of individual CA1 cellular activity in slices of hippocampus, using local applications of dopamine as reinforcement, has been reported (Belluzzi and Stein, 1983). Here we report blockade of such cellular operant conditioning by the dopamine antagonist chlorpromazine (CPZ). A single-barrelled glass micropipette for simultaneous recording and pressure injection was filled with dopamine (1 mM in 165 mM saline) and aimed at spontaneously active pyramidal cells in the CA1 layer of hippocampal slices. The neuronal response for reinforcement was a burst of relatively fast activity. To be eligible for reinforcement, such "bursts" had to contain a minimum number of spikes; this minimum number was individually established for each neuron studied so that, prior to operant conditioning, reinforceable "bursts" occurred at a rate of approximately 5 per minute. For reinforcement, the pressure injector was activated for 10-80 ms immediately after each "burst" to deliver a 10 µ-diameter droplet of drug to the cell. As shown in the Figure, neurons reinforced with dopamine exhibited significantly more "bursts" than controls reinforced with saline. When chlorpromazine (1 mM) was added to the dopamine solution (DA + CPZ), the reinforcing action of dopamine was abolished and the rate of "bursts" was suppressed below the saline control. On the other hand, neurons that received chlorpromazine alone (CPZ) exhibited the same number of "bursts" as those that had received saline. These results replicate our earlier reports of single-unit operant conditioning, and furthermore suggest that the reinforcing action of dopamine, which can be antagonized by CPZ in these experiments, is mediated at dopamine receptors.




(Supported by AFOSR Grant 84-0325)

256.9 RETARDATION OF CR ACQUISITION BY L-PIA: ANTAGONISM BY CAFFEINE AND THEOPHYLLINE. L. Winsky, J. A. Harvey and I. Gormezano, Psychology Department, The University of Iowa, Iowa City, IA 52242.

The adenosine receptor agonist $N^6$-(L-phenylisopropyl)adenosine (L-PIA) retards acquisition of the classically conditioned nictitating membrane response (NMR) of rabbits (Winsky et al., 1984, Soc. Neurosci. Abstr., 10, 793). The agonist activity of L-PIA has been associated with activation of $A_1$ adenosine receptors that produce decreases in brain levels of cAMP. This study examined the ability of the adenosine receptor antagonists caffeine and theophylline to block the effect of L-PIA on conditioned response (CR) acquisition in order to determine whether this effect of L-PIA is mediated by adenosine receptors. Rabbits received a subcutaneous injection of vehicle, caffeine (0.3 mmol/kg), or theophylline (0.2 mmol/kg) followed by vehicle or L-PIA (5.0 µmol/kg) prior to each of 10 days of acquisition training. Each daily (60 min) session consisted of 30 tone CS-shock UCS and 30 light CS-shock UCS pairings. Both caffeine and theophylline blocked the retardation produced by L-PIA as indicated by percent CRs, NMR onset latencies, and the number of trials required to attain the criteria of 10 consecutive CRs.

Caffeine and theophylline have been shown to inhibit brain phosphodiesterase in addition to blocking adenosine receptors. Thus, either or both of these actions may have produced a block of the L-PIA effect on acquisition. Since both of these effects of caffeine and theophylline would reverse the decreases in cAMP produced by L-PIA, we conclude that L-PIA retards CR acquisition by decreasing cAMP.

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256.10 EFFECT OF SP AND ITS FRAGMENTS ON MONOAMINE METABOLISM AND PASSIVE AVOIDANCE RETENTION. M. Pelleymounter, Q. Fischer*, K. Schlesinger*, M. Hall* and J. Stewart. Dept. Psych., Univ. Colo., Boulder, CO 80309 and Dept. Biochem., Univ. Colo. Schl. Med., Denver, CO 80262.

Several studies have shown that substance P (SP) enhances retention of avoidance learning paradigms in rodents, whether it is administered peripherally or intracerebroventricularly (icv). Recent work has also demonstrated that SP-induced memory enhancement is accompanied by increased striatal DOPAC/DA 1 hr after administration and training and increased nigral 5HIAA/5HT as late as 24 hr after injection and training. Since both behavioral and biochemical responses to SP can be detected many hours after SP administration (and probable enzymatic degradation), it has been suggested that the effects of SP may be mediated by fragments of the peptide rather than the complete molecule. There is evidence that central injections of N- or C-terminal fragments have distinct, and often opposing, behavioral effects. We have tested the effects of icv administration of SP, its N-terminal (1-7) fragment (SP-N), and its C-terminal (<E-SP [7-11]) fragment (SP-C), on passive avoidance retention and metabolite/monoamine ratios in the medial prefrontal cortex (MPC), striatum (STR), substantia nigra (SN), and raphe nucleus (R).

Polyethylene guide cannulae were implanted into the lateral ventricles of male Swiss-Webster mice. SP (1µg), SP-N (100 ng), SP-C (1µg) or phosphate-buffered saline (PBS) was injected into the ventricles in a volume of 5µl. One hr later, one group of mice was sacrificed and MPC, STR, SN, and R were dissected from frozen brain, using the tissue punch technique of Palkovits (Brain Res., 59:449, 1973). Brain punches were homogenized in 0.1N perchloric acid. Monoamine and metabolite levels were measured with reverse phase HPLC-ECD. The remaining group of mice was trained for a passive avoidance task. These animals were placed in a brightly-lit start-box, and latency to enter a darkened area of the apparatus was measured. Upon entry into the darkened area, mice were given a 0.4 mA, 5 sec footshock. They were immediately injected (icv) with SP, its fragments or PBS at the same doses used for the biochemical tests. Twenty-four hours later, latency to enter the darkened area was again measured. Retention was defined as Day 2 - Day 1 latency.

The only difference in monoamine/metabolite ratios was found in nigral 5HIAA/5HT, where SP-N and SP-C had opposing effects, with SP-N increasing 5HIAA/5HT over both SP and SP-C. SP-C significantly decreased 5HIAA/HT in comparison to PBS. SP-N also enhanced passive avoidance retention significantly better than did SP, SP-C, or PBS. These data support the idea that SP fragments (in particular the N-terminal fragment) may mediate the biochemical changes necessary to bring about some of the central effects of SP administration, such as enhanced retention of passive avoidance behavior.

256.11 EVIDENCE FOR A FUNCTIONAL INTERACTION BETWEEN SEROTONERGIC AND CHOLINERGIC MECHANISMS IN MEMORY RETRIEVAL. S.-O. Ögren*[1], W.S. Stone[2,3] and H.J. Altman[3] (SPON: E. Schoener). [1]Astra Läkemedel AB, S-151 85 Södertälje, Sweden; [2]Dept. of Psychology, Wayne State Univ., Detroit, MI 48202; and [3]Lafayette Clinic, Detroit, MI 48207.

Recent evidence suggests that there may be an interaction between the cholinergic (ACh) and serotonergic (5-HT) nervous systems. Alaproclate (ALAP), a 5-HT reuptake inhibitor, has been shown to potentiate the tremor induced by oxotremorine (OXO), a muscarinic ACh agonist, in mice. Both neurotransmitter systems are also believed to play a significant role in the processes underlying learning and memory. Previous studies from this laboratory have shown that independent administration of OXO or ALAP can enhance retrieval of a previously learned avoidance habit when administered shortly before retention testing. The purpose of the present study was to determine if a ACh X 5-HT interaction can also be demonstrated with respect to memory. Three experiments were conducted. The behavioral task used throughout was a standard two-compartment passive-avoidance apparatus in which the mice were tested for retention of the original avoidance habit 24 hrs. later. All drugs were administered i.p. either 30 or 60 min. prior to the retention test. In Experiment 1 the dose dependent effects of ALAP and OXO were assessed relative to saline injected controls. All drugs were administered 30 min. prior to the retention test. The results reaffirmed previous observations that OXO or ALAP significantly enhance retrieval of a previously learned avoidance habit in a dose-dependent fashion. In Experiment 2 we attempted to determine whether combined administration of OXO+ALAP would also enhance memory and if so what difference, if any, might be seen in the nature of the dose response curves. The results indicated that combined OXO+ALAP does enhance memory and that the entire dose response curve shifts significantly to the left. Finally, in Experiment 3 an attempt was made to block the memory enhancement induced by either OXO, ALAP, or OXO+ALAP. Previous studies from this laboratory have shown that the memory enhancement induced by ALAP is selectively blocked by quipazine (QUIP), while that induced by OXO is blocked by scopolamine (SCOP). However, the enhancement induced by ALAP is not antagonized by SCOP, and the enhancement induced by OXO is not blocked by QUIP. Therefore, in Experiment 3 animals were treated with either ALAP, OXO OR OXO+ALAP, and were pretreated with either SCOP or QUIP. Their performance during the retention test was then assessed. The results indicate that: QUIP blocked ALAP alone but had no effect on either OXO or the OXO+ALAP combination while SCOP blocked the effects of OXO alone but had only a slight effect on the performance of the ALAP or OXO+ALAP groups of animals. The results of the present study are taken as preliminary evidence for a functional interaction between the ACh and 5-HT nervous systems in memory retrieval.

256.12 FACILITATION OF DISCRIMINATION LEARNING IN THE RAT FOLLOWING CYTOTOXIC LESIONS OF THE SEROTONERGIC NERVOUS SYSTEM. H.J. Altman[1], H.J. Normile[1]* and S-O Ögren[2]* [1]Lafayette Clinic, Detroit, MI 48207, [2]Astra Läkemedel AB, S-151 85 Södertälje, Sweden.

Recent experimental evidence suggests that serotonin (5-HT) plays an important role in the processes underlying learning and memory. These suggestions are partly based on the results of animal studies which have shown that acute elevations of 5-HT activity on or about the time of behavioral assessment impaired learning. However, a complicated relation appears to exist between 5-HT depletion and learning and/or memory. For example, depletion of 5-HT by synthesis inhibitors or by cytotoxic or electrolytic lesions has been shown to either facilitate, impair or have no effect on the performance of animals in aversively motivated tasks. Several factors are likely to be responsible for these variable results including the nature of the behavioral task (e.g. passive avoidance, one- or two-way active avoidance), the methods employed to reduce 5-HT activity (e.g. p-chlorophenylalanine, indoleamine neurotoxins, electrolytic lesions) and/or the route of administration (e.g. central, peripheral). Moreover, the effects of 5-HT depletion on the performance of animals in positively reinforced tasks has not been examined. The purpose of the present study was, therefore, to determine what effects cytotoxic lesions of the 5-HTergic nervous system might have on a rat's ability to acquire a positively reinforced complex spatial discrimination (Stone 14-Unit T-maze). Lesions were made with the cytotoxin p-chloroamphetamine (PCA) which has been shown to have a long-term neurotoxic action on 5-HT neurons. The specificity of the PCA-induced effect was assessed by pretreating PCA-injected rats with the selective 5-HT reuptake blocker norzimeldine (NZIM). Four groups of male Sprague-Dawley rats were injected (8 and 7 days prior to the onset of behavioral assessment) as follows: Group 1 = SAL + SAL; Group 2 = SAL + PCA (2 x 10 mg/kg); Group 3 = NZIM (2 x 20 mg/kg) + SAL; Group 4 = NZIM (2 x 20 mg/kg) + PCA (2 x 10 mg/kg). The first injection preceded the second by 60 min. The rats were run 1 trial/day, 5 days/week for 5 consecutive weeks in the Stone maze using sweetened condensed milk as the reward. The total number of errors each rat made per trial was recorded. In addition, the total number of trials each animal took until it ran through the maze in one or less errors on two consecutive days (trials to criterion) was recorded. The results of this study suggest that 5-HT depletion can improve the acquisition of a spatial discrimination task. Specifically, PCA-injected rats (Group 2) made significantly fewer errors over days and required significantly fewer trials to reach criterion when compared to SAL controls (Group 1). Pretreatment with NZIM completely blocked the above effects. Surprisingly, Group 3 also exhibited significantly enhanced performance in the Stone maze.

256.13 THE EFFECTS OF SEROTONERGIC RECEPTOR BLOCKADE ON LEARNING AND MEMORY IN MICE. H.J. Normile* and H.J. Altman (Spon: J. Phillis). Lafayette Clinic, Detroit, MI 48207.

Recent experimental evidence suggests that serotonin may play an important role in the way information is processed by the brain. For example, experimental manipulations that enhance serotonergic (5-HTergic) neurotransmission have consistently been shown to interfere with the performance of animals in a variety of aversively motivated learning and memory tasks. On the other hand, the effects of lesions of the 5-HTergic nervous system are difficult to interpret mainly because seemingly complimentary experimental manipulations designed to reduce 5-HTergic activity have often produced conflicting and/or inconsistent results.

Recently, a number of highly potent and apparently selective 5-HTergic antagonists have been developed. We have previously reported that pre-test administration of pirenperone enhanced the memory of a previously learned aversive habit in mice (Normile and Altman, Neurosci. Abst. 10: 255, 1984). In contrast, Bammer (Neurosci. Biobehav. Reviews, 6: 247-296, 1982) has reported that administration of cyproheptadine (but not methysergide) prior to training impaired learning in rats. The purpose the present series of experiments was, therefore, to further assess the effects of 5-HTergic receptor blockade on learning and memory by administrating a number of 5-HTergic receptor antagonists (pirenperone, ketanserine or mianserine) either (1) prior to training, (2) following training, (3) prior to testing. The behavioral task used was a modification of the standard inhibitory avoidance paradigm (the lick-suppression task) in which thirsty mice were trained to avoid drinking from a tube by pairing shock with licks (Quartermain and Altman, Physiol. Psychol. 10: 283-292, 1982). All animals were tested 48 hours later under extinction (i.e., no shock) conditions and their latencies to complete 5 seconds of drinking recorded. Both dose- and time-dependent effects were examined.

The results of the study may be summarized as follows: Administration of each of the antagonists either following training or prior to testing resulted in a significant dose- and time-dependent elevation in the latency to complete 5 seconds of drinking at the time of testing. The elevated latencies could not be attributed to non-specific effects of the drugs on behavior as non-contingently trained mice injected with the highest dose of each drug failed to exhibit an elevation in their test latencies. On the other hand, the test performance of the mice injected with these same drugs prior to training was significantly impaired in both a dose- and time-dependent fashion. These data would appear to suggest a differential involvement of the 5-HTergic nervous sytem in the processes underlying learning and memory.

256.14 EFFECTS OF BETA-CARBOLINE-3-CARBOXYLIC ACID ETHYL ESTER ON THE RETENTION OF AVERSIVELY AND APPETITIVELY MOTIVATED TASKS IN RATS. L.J. Wichlinski, R.J. Tippy*, J.W. Hall*, S. Richmond*, R. Murray*, J. Wolfe*, D. Read*, and R.A. Jensen. Developmental Biopsychology Laboratory, Southern Illinois University, Carbondale, IL 62901.

Beta-carboline-3-carboxylate derivatives produce effects which are often directly opposite to those produced by benzodiazepines. Since benzodiazepines are well-known for their amnestic properties, we hypothesized that the beta-carboline-3-carboxylate derivative would produce an opposite effect, that is, enhancement of memory storage processes. Therefore, we investigated the effects of beta-carboline-3-carboxylic acid ethyl ester (B-CCE) on two different kinds of memory tasks in rats: an aversively motivated inhibitory avoidance task and an appetitively motivated T-maze water reward task. In the inhibitory avoidance task, male Sprague-Dawley rats (75-150 days of age) were injected i.p. with either 1.0 or 5.0 mg/kg B-CCE or saline immediately following exposure to a 0.6 mA, 1.0 sec foot shock. Three weeks later, retention was measured (600 sec ceiling). Median retention latencies were: Saline (n=19) - 466 sec; B-CCE 1.0 mg/kg (n=12) - 366 sec; B-CCE 5.0 mg/kg (n=16) -600 sec. Differences between the groups were statistically significant ($H=8.88$, $p<.02$). The latencies of the B-CCE 5.0 mg/kg group were significantly different from the saline group ($U=82.5$, $p<.05$), while the saline and B-CCE 1.0 mg/kg groups did not differ significantly from each other. The differences between the B-CCE-treated animals and controls were not due to nonspecific drug effects since in earlier pilot studies, unshocked B-CCE-treated rats (10.0 mg/kg) showed a median retention latency of 5.0 sec while shocked, B-CCE-treated rats showed a median retention latency of 600 sec.

In the appetitive task, water deprived rats 80-110 days of age were treated with either saline (n=15), B-CCE 1.0 mg/kg (n=15) or B-CCE 5.0 mg/kg (n=15) immediately following training to a criterion of three correct water-reinforced responses in the T-maze. Retention was tested 48 hr later by training the animals to a criterion of 5 correct responses. Eighty percent of the saline-injected rats entered the correct arm of the maze on the first retention test trial while 67% of the animals in each drug treatment condition did so. These differences were not statistically significant. Differences in total number of errors in the test session were also not significant ($F<1.0$). These results suggest that beta-carboline-3-carboxylate derivatives may facilitate memory storage processes for aversively but not appetitively motivated tasks in rats.

We thank the G.D. Searle Company for their donation of the B-CCE used in this research. Supported by a grant from the Office of Research, Development and Administration, Southern Illinois University at Carbondale.

256.15 REVERSAL OF AF64A-INDUCED MEMORY IMPAIRMENT BY CHOLINERGIC COMPOUNDS. A. Levy, R. Brandeis*, S. Dachir*, S. Luz*, Y. Karton*, E. Heldman*, Z. Pittel* and A. Fisher*, Israel Institute for Biological Research, Ness-Ziona, 70450, ISRAEL, and I. Hanin, University of Pittsburgh, Sch. Medicine, Pittsburgh, PA. 15213, U.S.A.

Biochemical studies have shown that intracerebroventricular injection of AF64A (3 nmoles/2ul/side) induces long-term selective reduction of choline acetyltransferase, paralleled by a significant decrease in acetylcholine (Fisher and Hanin, Ann. Rev. Pharmacol. Toxicol., in press). The cholinergic hypofunction induced by AF64A was accompanied by marked memory impairment, as demonstrated in several behavioral paradigms using rats.

In this study, attempts were made to reverse AF64A-induced cognitive impairment, using the cholinesterase inhibitor physostigmine and a selective central cholinergic agonist AF30 (Cohen and Fisher, US Patent 4,104,397, Aug. 1, 1978). Passive avoidance learning test was used, in which test-drugs were injected during the first minute post-training, followed by a retention test 24 hours later. Both physostigmine (0.06 mg/kg i.p.) and AF30 (1 mg/kg i.p.) were found to reverse the AF64A-induced memory impairment. The effects of these drugs on brain cholinergic markers were concomitantly monitored. AF64A injected rats were found to be impaired in two other memory paradigms. These two tests are: a) Eight-arm radial maze, in which drugs are injected following four free choices, and the test continued four hours later. b) Morris swimming test for three days, in which the drugs are injected following the first and the second day of training. The reversal of these behavioral impairments using the above drugs is currently under investigation.

The reversal of AF64A-induced memory impairment by cholinergic compounds strongly supports the idea that AF64A injected rats can be used: (i) to mimic the cholinergic hypofunction reported in Alzheimer's disease; (ii) in drug development studies aimed to ameliorate memory dysfunction in this disorder.

256.16 PAVLOVIAN-CONDITIONED MOTOR ACTIVITY PRODUCED BY D-AMPHETAMINE TREATMENT. P.M.Duncan and D.D. Hart*. Psychology Department, Old Dominion University, Norfolk, Va. 23508.

"Spontaneous" motor activity (SMA) responses to stimuli predicting d-amphetamine(DA) injection were investigated in a Pavlovian conditioning paradigm. Three groups of rats (doses=0,1.0,4.0mg/kg) were given IP injections on 6 alternate days, 30 min after being placed in a distinctive environment (Lafayette activity detectors), and remained there for an additional 30 min. SMA counts, indicative of locomotion, vigorous grooming and stereotyped behavior, were recorded at 10-min intervals both pre and post-inj. Pre-inj SMA was recorded in order to monitor acquisition of conditioned responses (CR) to the test apparatus as indicated by daily changes in SMA. On day 7 (test day) all rats received IP saline inj for measurement of CR to the injection procedure. Non-associative drug-produced SMA effects were controlled by DA inj given saline-grp rats 1 hr after being removed from the conditioning apparatus each day.

Mean Total SMA counts during 30-min pre/post injection periods

| Cond. Day | 1 | 2 | ....6 | Test Day |
|---|---|---|---|---|
| Sal Grp,n=12 | 1225/550 | 860/750 | 805/650 | 815/450 |
| DA 1 mg,n= 9 | 1030/1650 | 1180/2425 | 920/3150 | 860/750 |
| DA 4 mg,n= 9 | 960/3900 | 1060/3600 | 910/4200 | 630/1020 |

Significant($p<.05$) pre-inj differences occurred only in the 4 mg group,which increased in activity from cond days 1 to 2. Post-inj SMA increased significantly from cond day 1-6 in the 1 mg group. Test day post-saline inj SMA was greater in both drugged groups than in saline controls (combined drugged grps vs. saline grp, $p<.05$). Test day post-inj differences between the 2 drugged groups were not significant.

These results indicate that Pavlovian conditioning to place/apparatus stimuli occurred during the first cond day, especially in the high-dose grp. Subsequently, the injection procedure apparently gained most of the predictive power, since no pre-inj CR's were seen on the following cond days. CR's to injection-related stimuli were seen in both drugged groups on the test day, and were in the same direction, rather than compensatory to drug-produced responses. Visual observation suggested that these CR's consisted of locomotion, grooming, and (in the high-dose group) some amphetamine-stereotyped behavior.

256.17 DIAZEPAM (VALIUM) BLOCKS REFERENCE MEMORY, BUT NOT WORKING MEMORY, OF A RADIAL MAZE TASK. Mauro Caudarella and Robert Hough*. Dept. of Psychology, Queen's University, Kingston, Ont., Canada K7L 3N6.

Caudarella and others have shown that diazepam has impressive anterograde amnestic effects in aversively motivated, one-trial learning tasks: immediate "working" memory is unimpared but information held briefly in working memory apparently cannot be stored permanently while the animals are under the effects of diazepam; however, information already in long-term memory can be retrieved normally. Furthermore, the amnesia was not found to be "state-dependent". The fact that diazepam inhibits hippocampal circuits suggest that the hippocampus is involved in the long-term storage of information. On the other hand, some studies (e.g. Olton & Pappas, Neuropsychologia, 1979, 17, 669) have suggested that the hippocampus is involved only in working memory and not "reference" (long-term) memory in a radial arm maze. Even if diazepam's amnestic effects are not mediated principally by hippocampal circuits, it is clear that inhibition of the hippocampus by diazepam does not disrupt working memory. However, because aversively motivated one-trial tasks may represent a special case, the present experiment tested the effect of diazepam administered during acquisition on subsequent retention of a spatial discrimination problem using food reward presented in only 4 of the 8 arms (a random set for each rat) of a radial maze raised 50 cm off the floor. Four groups (n=7) of male Wistar rats were trained at 80% body weight to run the radial maze with a minimum of errors. An "error" was considered to be any "useless" entry into an arm that did not lead to food reward; errors can be of two types: first entries into a never baited arm constitute errors of reference memory while re-entries into a baited arm (now empty because the animal picked up the food some seconds before) or unbaited arm (still empty) constitute errors of working memory. 1) One group of rats received 1 mg/kg Valium Injectable® (DZ) 20 min. before each acquisition session (max. 10 min.) and, after a 3-day break, the proprietary vehicle (V) 20 min. before a single retention test with the same 4 arms baited. The other 3 groups completed a standard 2x2 design to check for "state-dependency": 2) acquisition-V, retention-V; 3) acquisition-DZ, retention-DZ; 4) acquisition-V, retention-DZ.
RESULTS: A. Working Memory Errors (max. 4). There were no significant differences (Mann-Whitney U, p>.1) among the 4 groups during the retention session. Thus, working memory was not impaired by diazepam. There were also no significant differences among the 4 groups in number of trials to criterion during the acquisition sessions. B. Reference Memory Errors (no max.; time limit-10 min.). Of all pairwise comparisons only one difference was significant in the retention test--group 1 vs. group 2; that is, only the rats that had received diazepam during acquisition showed significantly poorer retention, and furthermore, this memory deficit was not "state-dependent". Thus benzodiazepine receptors seem to be involved in the formation of long-term reference memory rather than immediate working memory.

## HUMAN BEHAVIORAL NEUROBIOLOGY I

257.1 THE MIND-BODY PROBLEM AS SUBSETS OF THE STRUCTURE-ACTIVITY RELATIONSHIP. B. E. Morton, Dept. of Biochemistry and Biophysics, University of Hawaii School of Medicine, Honolulu, HI 96822.

Much of neuroscience research deals both with physical structures and the activities of such structures. However, the important difference between the material properties of a structure, and its kinetic and thermodynamic properties (activities) has not been widely appreciated.

Biochemists, thinking that the mechanism of action of enzymes would be clarified if their structure could be determined, have now completely resolved the atomic structure of many such proteins. Yet, information about atomic positions in an unknown protein does not even permit the prediction that the molecule is an enzyme, much less what its catalytic activity might be.

Clearly in the case of enzymes, static physical structure is different than the dynamic kinetic and thermodynamic properties resulting from the action of such a structure. Yet, the activity of an enzyme is directly tied and exquisitely sensitive to perturbations in enzyme structural conformation. A lethal point mutation, for instance, which results in the substitution of only one amino acid out of the hundreds in the primary structure of an enzyme can totally eliminate catalytic activity.

At higher and lower structural levels in the universe similar relationships between structure and activity exist. Thus, at the levels of supramolecular assemblies, subcellular organelles, cells, tissues, organs, organisms, various groups of organisms, etc., activities exist that are absolutely dependent upon structure at that level, but like enzyme kinetics, are evanescent, matter-energy transformations.

At the level of the brain, such activities encompass what has been labeled as mind. The structural dependence of mind upon brain integrity is abundantly illustrated by the consequences of cerebral accidents or by the administration of psychoactive drugs. At the organism level, this structure-based activity is called life.

Therefore, just as enzyme structure vs. enzyme activity is a subset of the structure-activity relationship, so the mind-body problem is a misnomer that can be seen to represent subsets (at two different levels, ie., brain-mind and body-life) of the same structure-activity relationship.

The mind-body problem originated from the failure of body and brain structural data to account for mind. This anciently led to the creation of extracorporeal, supernatural solutions. Recently, more accurate understanding of the fundamental differences between structure and activity has permitted the solution of the mind-body problem as subsets of the structure-activity relationship.

257.2 A DISTINCT SPEECH PRODUCTION DEFICIT FOLLOWING DAMAGE TO THE LEFT SUPPLEMENTARY MOTOR AREA: EVIDENCE FROM TRANSCORTICAL MOTOR APHASIA. P.W.Schönle, Dept. of Clinical Neurophysiology, Center for Neurological Medicine, University of Göttingen, D-3400 Göttingen, West Germany

Several reports indicate participation of the supplementary motor area in speech production: 1) Penfield and Roberts (1959) have shown that electrical stimulation in and around the region of the supplementary motor cortex in man may result in arrest of ongoing speech or initiation of repetitive involuntary vocalizations. 2) Surgical ablation of the supplementary motor area produces transient speech disturbances with difficulty in initiating speech, anomia or mutism (Erickson and Wodsey, 1951).
3) Left anterior cerebral artery infarction involving the supplementary motor area leads to transcortical motor aphasia characterised by an absence of spontaneous speech, difficulty in initiating speech, near normal repetition and preserved auditory comprehension.

In the present study four patients with transcortical motor aphasia following damage to the left supplementary motor area were examined to delineate the level of their speech production deficit. Four patients with anomic aphasia, matched for sex and age, served as controls. The ability to name line drawings was taken as a baseline measure of speech production. Two experiments were designed to tap distinct levels of processing in speech production. In the first experiment subjects had to select pictures which were related semantically. In the second experiment pictures had to be selected whose names were related phonemically (pictures of homophones, e.g. yoke/yolk, or pictures of rhymes, e.g. bear/chair).

While patients with transcortical motor aphasia showed a severe deficit in overt speech production as indexed by picture naming, their ability to identify pictures with respect to semantic and phonemic relatedness was well preserved. The finding of intact phonemic knowledge about a given word in face of a severe speech production deficit indicates a distinct processing deficit at the level of translation of linguistic information into speech motor programs.

257.3 MULTIUNIT NEURONAL RESPONSES IN THE HUMAN MEDIAL TEMPORAL LOBE DURING A VERBAL RECENT MEMORY TASK. G. Heit, M.E. Smith, E. Halgren.
Brain Research Institute, UCLA; VA Southwest Regional Epilepsy Center, Los Angeles, CA 90024

Damage to the human Medial Temporal Lobe (MTL: hippocampus, parahippocampal gyrus, and amygdala) has been shown in clinical studies to lead to deficits in recent memory, while remote semantic memory is normal. Previous studies in our laboratory (Smith et al, 1984, Soc. Neurosci. Abs. 10:846) demonstrated that stimulus familiarity has profound effects on MTL field potentials evoked to words. These results prompted the current investigation of multiunit neuronal responses during verbal recent memory, face recognition, word/nonword discrimination, and object naming tasks. Subjects were instructed to keypress to targets consisting of either repeated or novel stimuli (recognition memory task), or to words rather than nonwords (lexical decision). In the naming task, the subject verbally responded with the name of a object portrayed in a line drawing. Electrode (40u fine wire) implantation and site selection were dictated on clinical grounds for the surgical management of medically intractable partial complex epilepsy.

The principal finding was that in contrast to the simultaneous recorded field-potentials cell firing in all areas was controlled primarly by overt behavioral responses. Cell-firing was largely insensitive to stimulus familiarity (novel vs repeat), stimulus type (verbal/nonverbal or meaningful/nonsensical), or task conditions (recent episodic vs remote semantic memory, namimg) Of the 38 units recorded, 29 demonstrated this firing correlate. Some units were excited for 100-250 msec beginning 200-500 msec before the keypress. Other units were activated both before and after, or entirely after the keypress for a period of 200 to 600 msec. The later responses had onset latencies of 50-100 msec post keypress. Brief (200 msec) inhibition followed by an equal period of excitation, or a long period (500 msec) of inhibition entirely following the behavioral response, were also observed.

These firing-patterns usually occured in trials with a behavioral output. However excitation or inhibition was occasionally seen in trials with no behavioral response. This excitation or inhibition was usually earlier in onset, briefer in duration, and smaller in amplitude then that occuring in trials with an overt behavioral response. Thus, although unit-firing occured predominately in those trials with keypress, sufficient firing occured during other trials to suggest that response-selection or decision-making rather than motor response per se is the essential behavioral correlate.
Supported by USPHS (NS18741) and the Veteran's Administration.

257.4 EVOKED POTENTIALS DURING LINGUISTIC PROTOTYPE FORMATION. J. G. Banghart* and R.M. Vardaris. Dept. of Psychology, Kent State University, Kent, OH 44242.

Previous studies of evoked potentials (EP's) during concept formation have yielded inconsistent results. Stuss and Picton (1978) failed to find EP's that were associated with different phases of learning. Peters et al., (1977) and Wilson et al. (1973), however found an overall increase in P300 amplitude over the course of learning. Wilson et al. attributed this change to increasing decision-confidence on the part of the Subjects (Ss).

For the present investigation, 10 right-handed male volunteers were first pre-trained on a paired-associates (PA) task to label nonsense syllables by pressing buttons.Then electrodes were applied to Pz, T3, and Oz. EP's were collected from these sites while Ss learned to categorize pseudo-random dot patterns as instances of a prototype pattern. After 90 trials, the prototypes were added to the list but no category names were supplied for them. As a control procedure, novel nonprototypes also were introduced. The EP's were averaged off-line. Trials containing eye-movement artifacts were not included.

Amplitudes and latencies were analyzed for P3(1) and P3(2) (post-stimulus positive peaks from 200-300msec and 300-400msec respectively). The following results were obtained (p .05). For P3(1)there were amplitude differences between correct instances and misclassified instances early in training, as well as between late misclassified instances and non-instances (controls). In addition there were corresponding latency differences between these response types. P3(2) amplitudes were greater for nonprototypes than for prototypes late in learning. Non-prototypes also had longer latencies early in training than prototypes late in training. Additional analyses will determine whether there are differences in these parameters for the three electrode sites.

It appears that P3(2) amplitude and latency were related to detection of prototype dot patterns in our study. In addition there were learning effects on P3(2). This late positive complex may index cogntive processing during the formation of prototypes.

257.5 SENSORY EVOKED EYEBLINK: ARTIFACT OR SIGNAL. M. R. Blackburn, L. J. Trejo* and G. W. Lewis. Navy Personnel Research and Development Center, San Diego, CA 92152.

Rejection of evoked potential (EP) records contaminated by obvious eyeblinks may not entirely solve the problem of eyeblink artifacts. Even when eyeblinks do not occur, signals with similar latency and polarity are present in the EPs. These signals may arise from small eye movements associated with eyeblinks, but may also occur alone when eyeblinks are suppressed. Our strategy was to compare EP trials that either included or were free from eyeblinks. Five subjects were presented with a simultaneous 90 dB (I) 1000 Hz tone (10 ms) and 10 ms 80 ftL checkerboard at 1 meter, repeatedly over 30 trials with a random ISI averaging 4 s. The EEG was recorded from frontal, parietal, and central sites for 1000 ms pre-and 1000 ms post-stim. Although subjects were instructed to suppress eyeblinks, they often blinked involuntarily following stimulation. Single trials were edited for eyeblinks (Fp1-Cz). We inferred eyeblinks from the presence of a positive transient in the post-stimulus EEG that was greater than twice the maximum peak-to-peak amplitude of the pre-stim activity (20-40 μv). Trials free of eyeblinks were identified by the absence of a positive transient exceeding the pre-stim peak-to-peak amplitude. Eyeblink trials were averaged and compared with an equal number of eyeblink-free trials that occurred in close temporal proximity to the eyeblink trials. We found that most involuntary eyeblink potentials occurred within a narrow window centered about 100 ms post-stim. These potentials were recorded at all sites when referenced to the ipsilateral ear. In the parietal to Cz derivation, EP waveforms from trials with and without eyeblinks were similar. In particular, both types contained the sharp transient positive peak with the latency characteristic of the eyeblink, and a negative deflection at about 200 ms. The possibility that small eye movements contribute to these potentials in the absence of eyelid closures cannot be excluded. Averaged power spectra of the pre-stim epochs showed that eyeblinks were preceded by less alpha activity than non-eyeblink trials. Thus, higher states of cortical arousal appear to exist during eyeblink trials than during non-eyeblink trials.

257.6 EVIDENCE FOR PRECORTICAL GATING DURING SELECTIVE ATTENTION IN HUMANS. M. Oakley*, R. G. Eason, R. Moore* & S. Conder* Dept. of Psychology, U. of N. Carolina, Greensboro, N. C. 27412.

Both behavioral (Posner, et al., In Pick, (Ed). Erlbaum, Assoc., 1977) and electrophysiological data (Eason, B. Psychon. Soc., 1984) suggest that when one selectively attends to a location in space where a task relevant stimulus is expected to appear, neural pathways are biased such that impulses are preferentially transmitted over them. Whether the biasing occurs at a precortical or cortical level, or both, remains unresolved. Eason and assoc. (Physiol. Psych., 1983; B. Psychon. Soc., 1984) have reported that selective attention to specific locations alters neural activity in the visual pathways at the level of the retina. They interpreted their findings as evidence for attention-induced precortical gating in humans. Lukas (Int. J. Neurosci., 1981) has reported similar effects at the level of the cochlear nucleus for the auditory system. Hillyard and assoc., however, have been unable to find evidence for precortical gating in either of these sensory modalities (Hillyard & Kutas, Ann. Rev. Psychol., 1983; Mangun, et al., Neurosci. Abstr., 1984).

In view of continuing uncertainty over whether precortical gating occurs in humans, we have conducted further studies bearing on this issue. Using the same paradigm as in previous studies wherein subjects responded to target stimuli appearing at a given spatial location in the right or left visual field, we were able to modulate the amplitude of short-latency components of scalp-recorded VERs as a function of the spatial attention manipulation (Oakley & Eason, EPA Proc. & Abstr., 1985). The earliest attention-sensitive component, most clearly manifested at frontal scalp locations, had onset-offset latencies of approx. 30-60 msec. Deflections with onset-offset latencies of 60-110 and 110-150 msec. also were affected at frontal as well as at more posterior scalp locations. Based on latency data obtained from single unit recordings from monkeys (Wurtz & Assoc., Nat. Eye Inst.) and cats (Baker, et al., J. Neurophysiol., 1969), it seems probable that the 30-60 msec. component, and perhaps also the 60-110 msec. component, constitutes a far-field recording of precortical activity.

We presently are conducting further studies in an effort to corroborate and extend these results. Data have been obtained from four subjects while working under the instruction to make either a finger lift response or an eye saccade to the location at which the target stimulus appears in the relevant visual field. Based on VERs obtained from $Fp_2$ and $F_4$ over eight sessions involving a total of 6400 flash presentations per subject, three of four subjects have shown an enhanced negativity in the 20-70 msec. range when a given spatial location is attended. These data provide further evidence for precortical gating during selective attention to specific spatial locations.

257.7 BRAIN PERFUSION IN SCHIZOPHRENIA: A DYNAMIC CT STUDY. T.W. Kruckeberg[1], E.M. Burns[1], H.A. Nasrallah[2], M.H. Kathol[3], S.M. Chapman[4].
[1]College of Nursing, University of Iowa, [2]Department of Psychiatry, Ohio State University, Columbus, OH 43210, [3]Department of Radiology, University of Iowa Hospitals & Clinics, [4]Department of Psychiatry, Veterans Administration Hospital, Iowa City, IA, 52242 USA.

Dynamic brain scans were performed on ten consenting right-handed males (mean age 36 years) fulfilling DSM III criteria for chronic schizophrenia using a Picker 1200SX CT scanner. Subjects had a drug wash-out for 10-14 days prior to scanning.

A series of 30 rapid-sequence scans of a pre-selected transverse brain section (15 degrees from the canthomeatal line) were obtained, 24 of which followed the injection via a mechanical injector of 32ml of Hypaque 75 (at a rate of 8ml per sec). Graphs of changes in brain density over time in ten bilateral brain regions were generated by computer.

Peak contrast-enhanced density, arterial mean transit time (AMTT), capillary mean transit time (CMTT) and corrected capillary mean transit time (CCMTT) were examined for right-left differences and any anterior posterior (AP) gradient changes. Asymmetry of peak contrast-enhanced density was detected in the right versus left middle temporal cortex, cerebellum and cerebellar vermis. Either a flattened or hyper-posterior AP gradient was observed in all of the subjects. Fifty percent of the sample manifested prolonged CCMTT in one or more brain regions. Evaluation of these findings relative to brain perfusion will be discussed.

This work was supported by NIH grant MH38514 and VA Hospital, Iowa City, Iowa.

257.8 PATTERNS OF MEMORY FAILURE AFTER SCOPOLAMINE TREATMENT: IMPLICATIONS FOR CHOLINERGIC HYPOTHESES OF DEMENTIA. D.S. Janowsky, W.W. Beatty and N. Butters. VA Medical Center and Department of Psychiatry, UCSD School of Medicine, San Diego, CA 92093

Severe loss of both anterograde and retrograde memory is characteristic of both Huntington's disease (HD) and Alzheimer's disease (AD). In both forms of dementia, marked loss of cholinergic neurons (in the caudate nucleus and the basal forebrain respectively) constitute an important feature of the neuropathology. Since scopolamine treatment in normal subjects interferes with new learning and retards retrieval of information from semantic memory on verbal fluency tests, it has been proposed that scopolamine provides a useful drug model of the memory disturbance in AD (Bartus, et al., 1982) or HD (Caine, et al., 1981). Although the memory deficits in AD and HD appear grossly similar on recall tests, there are marked differences in the pattern of memory failure. Specifically, AD patients exhibit a marked tendency to make perseverative responses, but HD patients do not. The use of recognition procedures differentially improves memory performance in HD but not in AD, although both groups of patients make more false positive responses. If scopolamine provides a useful model of memory loss in dementia, then the pattern of memory failure after scopolamine should mimic that seen in AD or HD.

To test this idea we compared the effects of 0.5 mg scopolamine, 0.1-0.2 mg glycopyrrolate (a peripherally active anticholinergic), or saline on the performance of healthy male volunteers (Age: 20-32 years) on a battery of memory tests. Treatments were given in a counterbalanced order using a within-subjects design. Scopolamine (but not glycopyrrolate) retarded recall and tended to impair recognition of a 14-word list, but the rate of false positive errors was not affected. Scopolamine (but not glycopyrrolate) also impaired recall on the Brown-Peterson short term memory test, but the proportion of perseverative errors was not altered. Neither scopolamine nor glycopyrrolate affected verbal fluency or learning on a symbol-digit paired associate task. Performance on all of these measures is seriously disturbed in HD and AD.

Although scopolamine does impair memory, the pattern of memory failure after drug treatment in the dose used does not mimic that seen in HD or AD. Hence, this pharmacological agent may not provide as useful a model of memory loss in these forms of dementia as previously believed. The present results are not supportive of the hypothesis that the loss of central muscarinic circuits are fundamental to the memory disturbances of HD and AD.

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257.9 MULTI-CHANNEL EEG COHERENCE ANALYSES DURING A CONTINUOUS MOTOR TASK. M.R.Ford*, D.K.Dekker* and J.W.Goethe* (SPON: R.B.Wallace) Research Department, Institute of Living, Hartford, CT 06106.

EEG coherence (COH), based on autospectral and cross-spectral calculations, is a frequency-specific, phase independent, linear index of the degree of coupling of two periodic phenomena. Previous reports from our laboratory (Scammon, M.E., Beh. Res. Meth. Inst., 13:517, 1981) suggest that high COH between two brain regions implies a functional connectivity between them, where one region is driving the other, or where both are being driven by a third source. Given that there is increased involvement of frontal, premotor and sensorimotor cortex during consciously directed movement, we proposed to investigate correlative changes in COH recorded from left and right prefrontal, premotor, sensorimotor and parietal regions during continuous movement conditions. Fourteen right-handed women (ages 18-39; $\bar{x}$ = 26.7 years) were instructed to alternate continuously between fist clenching and finger extension of the right hand, left hand, both hands, or neither hand (rest condition) in a counter-balanced sequence (4 one-minute trials for each condition; 16 total minutes). One minute each of intentional eye movement (EOG) and intentional facial muscle tension (EMG) were recorded for artifact assessment and control. Eight channels of eyes-closed EEG were recorded from $F_{p2}$, $F_3$, $F_4$, $C_3$, $C_4$, $P_3$ and $P_4$, each referenced to the ipsilateral earlobe. FFT spectral power analyses (POW) were conducted (sampling rate = 128 points/second; PDP-11/23) on 8 EEG channels, and COH analyses (percentage of seconds/minute in which COH $\geq$ .80) were performed on 16 pairs of leads: 4 interhemispheric, 6 intrahemispheric (left), and 6 intrahemispheric (right). One Hertz ($H_z$) interval data were combined to form frequency bands of 9-12$H_z$, 13-17$H_z$, and 18-31$H_z$ (slower frequencies were not analyzed due to the high probability of artifact contamination). Results show consistent and frequently significant increases in 9-12 $H_z$ COH during all movement comparisons except those recorded from parietal regions. Absolute POW levels in the 9-12$H_z$ band were unchanged from resting levels over all subjects; however, low-POW subjects showed consistent and significant increases in 9-12$H_z$ POW during movement, while high-POW subjects showed decreases in 9-12$H_z$ POW. Significant, though less consistent lateralized effects were observed. Significant increases in 13-17$H_z$ and 18-31$H_z$ COH were found only in premotor and sensorimotor regions. POW was unchanged for both "beta" bands. Results were independent from EOG, EMG, or SMR (sensorimotor rhythm) influences, and the COH increases were not present in adjacent parietal regions. The results strongly suggest that COH, especially in the 9-10$H_z$ range, is integrally involved in the execution of a continuous motor task, and support the contention that COH may represent the electrophysiological correlate of functional connectivity, or communication, among brain regions.

257.10 PRESERVED ENCODING CAPACITY IN PATIENTS WITH HUNTINGTON'S DISEASE. N. Butters and W.W. Beatty. VA Medical Center and Department of Psychiatry, UCSD School of Medicine, San Diego, CA 92161.

Huntington's Disease (HD) is a genetically transmitted basal ganglia disorder characterized by involuntary choreiform movements and a progressive dementia. Among the earliest and most prominent signs of the HD patients' dementia are anterograde and retrograde memory deficits. Some studies concerned with the cognitive processes underlying the HD patients' memory deficiencies have emphasized failures in encoding whereas others have concluded that failures in retrieval play a primary role.

To re-examine encoding in HD we studied 12 patients and 12 controls that were closely matched in age, gender and education. In the first experiment subjects learned a 14-word list containing 7 highly imageable words and 7 words that were difficult to image. Controls displayed superior immediate and delayed recall and better delayed recognition, but both groups remembered more highly imageable words. The magnitude of this effect was comparable for HD patients and controls and evident even on the first recall trial. In the second experiment using the same subjects we examined the buildup of proactive interference (PI) and its release following a taxonomic shift. Controls recalled more words than did HD patients, but both groups exhibited comparable release from PI. These findings demonstrate that encoding capacity is relatively well preserved in HD. Hence encoding deficits are not likely to be a major source of defective memory in this disorder.

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257.11 EVENT-RELATED BRAIN POTENTIALS REVEAL PROCESSING GRADIENTS DURING VISUAL-SPATIAL SELECTIVE ATTENTION IN MAN. G.R. Mangun* and S.A. Hillyard. Department of Neurosciences, UCSD, La Jolla, CA 92093.

Selective attention to a region of the visual field has been likened to a "spotlight" that facilitates the processing of stimuli falling within its boundaries. Recent work suggests that spatial attention is not focussed upon a particular region in a discrete all or none fashion, but rather falls off continuously as a function of distance from the attended locus (i.e., a spatial gradient of attention). The possibility that spatial attention produces facilitated processing of stimuli in the vicinity of an actively attended location can be investigated using event-related brain potentials (ERPs). ERPs have been used to demonstrate electrophysiological correlates of selective attention and allow analysis of the processing of both relevant and irrelevant events. We investigated the spatial distribution of attentional gradients using ERPs to index the processing of irrelevant probe stimuli at varying distances from the attended locus.

Subjects viewed a video display that presented rectangular flashes at multiple locations in the visual fields. For each stimulus in one half-field, another stimulus was located in the mirror image location in the opposite half-field. The stimuli flashed one at a time, in random order, with ISIs ranging between 300-600 msec. Subjects attended to one stimulus location during each experimental run in order to detect infrequent target flashes that appeared at that location 15% of the time. ERPs for each of the flashing stimuli were recorded bilaterally from multiple scalp sites.

Selective visual-spatial attention was evident as an enhancement of the N170 and N270 components over frontal, central, parietal and occipital scalp sites for the attended stimuli as compared to when they were inattended (i.e., during attention to the homologous location in the opposite field). Gradient effects on ERP amplitudes were evident for unattended, irrelevant probe stimuli that were located in spatial proximity to the attended stimuli. Depending upon the stimulus configuration, the gradient effects were seen as enhancements of the P110, N190 and P250 components of the ERP to the probe stimuli.

The results are interpreted as supporting a continuous gradient structure in visual-spatial attention rather than a discrete spotlight.

257.12 SINGLE NEURON ACTIVITY IN THE HUMAN TEMPORAL LOBE: I.LISTENING AND SPEAKING. by O. Creutzfeldt*, G. A. Ojemann and E. Lettich*(SPON: A. A. Ward Jr.). Dept. of Neurol. Surgery, Univ. of Washington, Seattle, WA. 98195 and Dept. of Neurobiol., Max-Planck-Institute of Biophysical Chemistry, Göttingen, West Germany.

We have recorded single and multiple unit activity (with lacquer insulated tungsten microelectrodes) from various locations in the temporal lobe of consenting awake human subjects during epilepsy surgery. Recordings were restricted to regions which would subsequently be resected.

The patients listened to 20 multi-syllable words each preceded by a 1000Hz pulse. During a subsequent presentation they had to repeat each word. In the right and left superior temporal gyrus, below the face area, neuron activation occurred throughout the word sound, but sometimes was restricted to the second part of a composed word (e.g. airplane) with less activation to single multi-syllable words (e.g. alligator). Other neurons showed little or no activation during the word presentation but strong activation when the patient repeated the word. Activation during listening or speaking were typically accompanied by a surface negative potential in the averaged ECoG. In the middle temporal gyrus a few neurons also showed a strong activation during word repetition or a variable activation during the preceding word presentation. But most neurons there showed a strong suppression during listening and/or the word repetition. This suppression was typically accompanied by a broad surface positive potential in the averaged ECoG, on which a negative wave may be superimposed if neurons in this region were excited during the word repetition phase. All neurons responded only a little to a 1000Hz beep alone or to abrupt or continuous noises in the operating room. No speech related activity was found in the inferior temporal gyrus recordings.

These findings demonstrate that activity related to speech perception and production can be recorded in the superior and middle temporal gyri of both hemispheres outside the primary auditory cortex and the typical language areas. The activity may be specifically related to certain parts of a word with semantic significance.

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257.13 SINGLE NEURON ACTIVITY IN THE HUMAN TEMPORAL LOBE: II.: NAMING, READING, MEMORY, FACE AND FIGURE MATCHING. G. Ojemann, O. Creutzfeldt* and E. Lettich*. Dept. of Neurol. Surgery, Univ. of Washington, Seattle, WA. 98195 and Dept. of Neurobiol., Max-Planck-Institue of Biophysical Chemistry, Gottingen, West Germany.

Single and multiple neuron activity was recorded during epilepsy surgery from temporal cortex (that was subsequently resected) of consenting awake patients while the patient performed various visually presented cognitive tasks.

Recordings from the left hemisphere were made during naming, reading and verbal memory tasks, with spatial matching tasks using the same items as controls. In the left superior and middle temporal gyri, neurons showed activation during several measures of silent and overt object naming and word reading compared to the spatial task controls. The same neurons also showed changes during speaking and listening to words (activation or suppression). In left inferior and middle temporal gyri an enhancement of neuron activity was observed during verbal memory input (silent naming of an object to be remembered) and with the reading task during which the memory was stored, while activity was reduced during silent or overt naming and reading that were not part of a memory task as well as during the spatial task controls. Neuronal responses were variable between trials, but in one example this response variability was related to a concomitant variability of performance. Inferior temporal neurons did not change during speaking and listening.

Recordings from the right hemisphere were made during face and figure matching, naming and labeling of facial emotional expressions. In the right superior and middle temporal gyri neurons were activated during matching tasks, compared to the other tasks; on occasion this activation was greater for face than complex figure matching. Some of these neurons also showed changes during speaking and listening.

The functional relationship of recording sites to the same tasks was independently established by electrical stimulation mapping. In some, but not all instances, maximal neuronal firing increases occurred during those tasks related to the recording site by stimulation mapping, including cases with changes during both mapping and recording related to input to verbal memory and face matching. Our findings suggest that neurons in various portions of temporal lobe may be differentially involved in silent and overt naming and reading, memory acquisition and spatial matching tasks.

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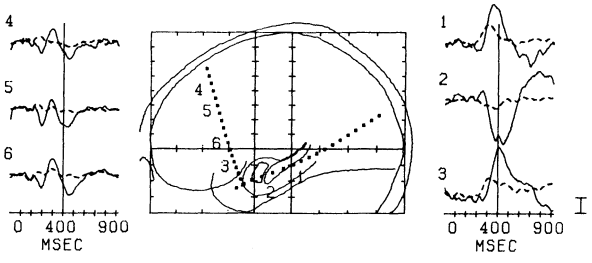
257.14 A POSSIBLE FRONTAL LOBE CONTRIBUTION TO SCALP P300. C.C. Wood and G. McCarthy, VA Medical Center, West Haven, CT and Yale University School of Medicine, New Haven, CT 06516

Human intracranial recordings provide an important source of evidence concerning the generators of P300 and other scalp ERPs. To date the only intracranial concomitants of scalp P300 with voltage gradients suggesting local origin have been reported in medial temporal lobe structures, in particular the hippocampus and amygdala (Halgren et al., Science, 1980, 210: 803-805; McCarthy et al., Soc. Neurosci. Abs., 1982, 8: 976; Wood et al., Ann. N.Y. Acad. Sci., 1984, 425: 681-721). Here we describe task-related ERPs recorded from locations throughout the human frontal lobe that could also contribute to scalp P300.

ERPs were recorded from as many as 8 18-contact depth probes in patients undergoing evaluation for possible epilepsy surgery. Subjects performed a series of two-choice categorization tasks in which stimuli occurred randomly with probabilities of .8 and .2 and subjects counted occurrences from the infrequent stimulus category. In different tasks, the stimuli to be categorized were auditory, somatosensory, or visual.

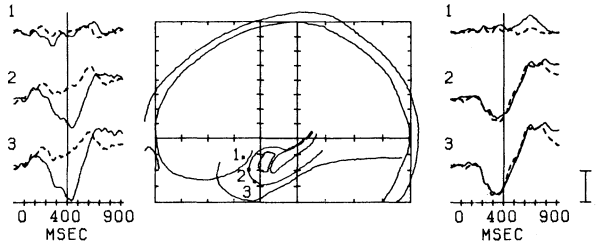
At locations in the medial temporal lobe, the previously reported ERP concomitants of P300 were obtained: A positivity posterior to the hippocampus, a sharp negativity in or near the hippocampus, and a positivity anteriorly in the region of the amygdala (4-6 in Fig; solid lines are ERPs for counted soft clicks, P=.2; dotted lines are ERPs for loud clicks, P=.8; cal: 50uV; positive up). In the frontal lobe a diffusely distributed negative-positive-negative sequence was obtained which began earlier than but overlapped with the temporal lobe activity (1-3 in Fig.). In some patients, the frontal potentials inverted across frontal cortex, suggesting a frontal cortex origin for at least part of the frontal activity. These data emphasize the likelihood of multiple generators of scalp P300.



257.15 HUMAN INTRACRANIAL ERPs DURING LEXICAL DECISION. G. McCarthy, C.C. Wood and S. Bentin, VA Medical Center, West Haven, CT and Yale University School of Medicine, New Haven, CT 06516

We have recently described intracranial event-related potentials (ERPs) recorded from the medial temporal lobe during sentence processing in humans (McCarthy and Wood, Soc. Neurosci. Abs., 1984, 10: 847). Large negative potentials with peak latencies of about 400-500 msec were obtained from electrodes in the region of the amygdala to sentence endings that were semantically anomalous, but not to normal sentence endings. We now report that potentials of similar polarity, latency, and intracranial distribution are obtained during a lexical decision task. The task was identical to that described by Bentin et al. (Electroenceph. Clin. Neurophys., 1985, 60: 343-355): 240 words and 240 nonwords were randomly presented one word per trial and subjects were required to indicate whether each stimulus was a word or not. Nine patients were studied, each of whom had chronic implanted electrodes for evaluation of possible neurosurgery to relieve intractable epilepsy.

The figure compares waveform morphology and intracranial distribution of the negative ERPs in the anomalous sentence (left) and lexical decision tasks (right). In the sentence task, anomalous endings (solid) elicited a large negativity in the vicinity of the amygdala which was absent for normal endings (dashed). In the lexical decision task, both nonword (dashed) and word stimuli (solid) elicited potentials with similar morphology and distribution to those in the sentence task. Thus, lexical decision and anomalous sentence tasks appear to elicit similar activity in the anterior medial temporal lobe. Whether such activity differs for primed and unprimed words is currently being investigated. (Calibration: 50 uV, positive upwards).




## SPINAL CORD AND BRAINSTEM III

258.1 2-DEOXYGLUCOSE UPTAKE IN THE CERVICAL CORD OF THALAMIC CATS DURING FORELIMB STEPPING ON A MOTOR-DRIVEN TREADMILL. M. Shimamura*, V.R. Edgerton, I. Kogure* and T. Fuwa* (SPON: F. Zajac). Dept. of Neurophysiology, Tokyo Metro. Inst. for Neurosci., Tokyo 183 Japan, and Dept. of Kinesiology, UCLA, Los Angeles, CA 90024.

Tracer amounts of [$^{14}$C]-2-deoxy-D-glucose (2-DG) were infused to detect areas of the cervical cord involved in forelimb stepping in thalamic and low spinal cats. Under ether anesthesia, cats were transected at stereotaxic A12 level and Th10 segment. Immediately after transection ether was withdrawn. Approximately one hour later, 100μCi/kg of 2-DG was infused into the jugular vein and one of three procedures were followed: 3 cats stepped on a motor-driven treadmill, 3 were placed on the treadmill with the belt stationary (extensor rigidity), and 2 were anesthetized with sodium pentobarbital. In all experiments 45 minutes elapsed between the time of injection of 2-DG and when cardiac arrest was induced by KCI injection, after which the brain and spinal cord were quickly removed and frozen in 2-methybutane cooled with dry ice. Frozen sections, 60μm thick, were cut, placed on glass slides, air dried, and placed on x-ray film. The film was processed after about seven days of exposure. Optical densities of the autoradiograms corresponding to specific anatomical structures at about C2 and C7 were determined. For anatomical localization, autoradiograms were compared with counterstrained histological sections. Forelimb stepping led to a discrete region of increased 2-DG uptake dorsomedially in the dorsal horn, predominantly in Rexed's laminae II-IV and in intermediate zone VII. A less marked increase in labeling was observed in the white matter at C6-C7 segments. The ventrolateral and dorsolateral funiculus exhibited slightly higher 2-DG uptake than the dorsomedial and ventromedial funiculi in both stepping and rigid cats. The ratio of uptake in the gray and white matter in C7 was considerably higher in stepping than in rigid cats. A less substantial difference between the gray and white mattter was noted at C2. The 2-DG uptake rate in anesthetized cats was lower at both C2 and C7 than in either the rigid or stepping cats. The most distinctive difference in all cats was the greater uptake around the outer rim than the more central regions of the dorsal horn. This contrast was the highest in the rigid and stepping cats. The regions of elevated 2-DG uptake may have been due to increased afferent discharges of the forelimb and its ascending volleys as well as to descending input from the brainstem.

258.2 ELECTROPHYSIOLOGICAL CHARACTERISTICS OF LONG-LOOP REFLEX COMPONENTS IN LEG MUSCLES IN MAN DURING VOLITIONAL, POSTURAL AND GAIT ACTIVITY V. Deletis,* M. R. Dimitrijevic, A. M. Sherwood, Dept. of Clinical Neurophysiology, The Institute for Rehabilitation and Research and the Dept. of Rehabilitation, Baylor College of Medicine, Houston, TX 77030

Electrical stimulation of the median nerve at the wrist has been shown to generate segmental and long-loop reflex responses. Stimulation of the superficial radial nerve at the wrist generated only long-loop reflex responses. However, these responses to stimulation of either nerve were present in healthy subjects only when accompanied by concomitant volitional activity of the involved muscle (Eisen et al., EEG clin Neurophysiol 59:205-213, 1984). The size of the long-loop reflex response can be increased by performing dynamic, skillful movements instead of applying steady, sustained activity during stimulation (Conrad and Aschoff, EEG clin Neurophysiol. 42:107-116, 1977). Initial findings were interpreted to mean that the long-loop reflex response is mediated via the spino-bulbospinal pathway while later results suggested the importance of the spino-corticospinal pathway.

We studied the effects of various maneuvers on segmental and long-loop reflex components recorded from leg muscles in healthy adult subjects. These reflexes were elicited by peroneal nerve stimulation together with tonic volitional activation of the involved muscle or repetitive movement of the ankle while in the sitting position. We also studied these reflexes in the standing position, activating the involved muscle by standing on the heels, through postural disturbances, or walking in place while stimulating. We were able to modify the segmental reflex response in the lower extremities through the use of these maneuvers, but we were not able to modify the long-loop reflex systematically, as has been found in the upper extremity. In light of the proposed mechanisms of the long-loop reflex in the upper extremity, one might expect to find similar reflex characteristics in the lower extremities. However we did not find selective facilitation of the long-loop reflex component in the lower extremity. Further studies are needed to clarify the differences between upper and lower extremity responses, which may in turn yield new insights into the mechanisms involved in the long-loop reflexes.

258.3 RHYTHMIC DEPOLARIZATIONS OF LUMBO-SACRAL AND CERVICAL DORSAL ROOTS DURING FICTIVE LOCOMOTION IN THE CAT. R. Dubuc, J. -M. Cabelguen* and S. Rossignol. Centre de recherche en sciences neurologiques, Département de physiologie, Université de Montréal, Montréal, Québec, Canada H3C 3J7.

During locomotion, the reflex responses to cutaneous input are subjected to phasic amplitude modulation. There are several levels at which such modulation could occur, the first of which is the primary afferent terminals themselves. Fifteen cats were decorticated under methohexital sodium anaesthesia (10 mg/Kg) and paralysed. Muscle nerves of either the hind- or the forelimbs were recorded during fictive locomotion. Proximal stumps of cut dorsal roots were recorded in a pool of warm paraffin oil. Dorsal root potentials (DRPs) showed clear fluctuations at the periodicity of the locomotor cycle as reported by Bayev and Kostyuk (Neurosci., 7, 1982, 1401-1409). However, no DC shifts were observed at the onset or offset of the locomotion bouts. Typically, the cervical or lumbo-sacral DRPs showed two peaks of depolarization per cycle. For instance, in the lumbar cord, the first depolarization peak (N1) occurred near the maximum of flexor activity (Sartorius lateralis) whilst the second (N2) appeared at the maximum of activity in the extensor muscle nerve (Semimembranosus anterior). Following each depolarization peak, there was a sharp trough of hyperpolarization. The first one (P1) appeared after the onset of extensor activity at a time wich could correspond to foot contact in a walking cat with similar cycle durations. The second (P2) occurred near the onset of flexor activity; this time relationship with the flexor activity strongly suggests a relation to foot lift-off. Analyses showed that the N1 and P1 components are related to flexor activity since, as the flexor burst, they do not vary with changes in cycle duration. On the other hand, N2 and P2 showed a strong correlation with the latter, suggesting that they are related to the extensor activity. It was also clear that the two depolarization peaks in the DRPs were related to the ipsilateral cycle and therefore, that N2 was not a reflection of the contralateral flexion. It was of interest to find in those preparations that many units in cut dorsal root filaments showed rhythmic discharges at the periodicity of the step cycle. Moreover, several such units showed antidromic discharges at phases of the step cycle corresponding to N1 or N2. It thus appears that the locomotor network exerts complex presynaptic effects on the primary afferent terminals which are reflected by two distinct periods of depolarization per step cycle. However, because DRPs represent presynaptic events occurring in several types of afferents, it is not yet possible to establish their relationship with the modulation of specific cutaneous reflex pathways. (Supported by the Canadian MRC).

258.4 FEATURES OF ENTRAINMENT OF SPINAL PATTERN GENERATORS FOR LOCOMOTOR ACTIVITY IN THE LAMPREY. A.D. McClellan & K.A. Sigvardt, Dept. of Physiology & Biophysics, Univ. of Iowa, Iowa City, IA and Dept. of Neurology, Univ. of Cal.-Davis, VAMC, Martinez, CA.

Central pattern generators (CPGs) in the in vitro lamprey spinal cord generate "fictive" swimming motor patterns. Mechanosensitive elements within the spinal cord are sensitive to movement of the spinal cord/notochord (Grillner, McClellan & Sigvardt, 1982; Grillner, Williams & Lagerback, 1984) and rhythmic bending of the notochord can entrain the burst pattern (Grillner, McClellan & Perrett, 1981). The intraspinal mechanosensitive edge cells, which are located in the lateral white matter, are thought to mediate the entrainment effect on the CPGs. We did a series of experiments to determine the characteristics of this movement-related feedback and how this feedback is distributed to the CPGs.

The spinal cord/notochord was dissected from adult silver lampreys, Ichthyomyzon unicuspis. A 20-30 segment piece was pinned down in the recording dish, usually with the caudal few segments free and attached to a pen motor which moved the end of the cord from side-to-side, mimicking movements that would occur during swimming. Swimming motor activity was elicited by bath application of 0.25-1.0 mM D-glutamate and motor activity was recorded from ventral roots. The dorsal roots were usually cut.

During entrainment at different frequencies, the burst duration and phase lag plots versus rhythm frequency were similar to those observed during actual swimming. Imposed step movements activated mechanosensitive elements, reset the swimming rhythm, and depressed ipsilateral or enhanced contralateral ventral root bursts. Increasing the amplitude of rhythmic imposed movements resulted in a gradual increase in the range of frequencies over which entrainment was effective.

In a low calcium Ringers solution, imposed lateral bending elicited ascending and descending activity in lateral spinal fascicles as far away as 12-24 segments from the point of bending. If the portion of spinal cord caudal to and including the point of bending was bathed in either a normal or low calcium Ringers solution, entrainment of the locomotor rhythm in more rostral segments was blocked even though activity from the mechanosensitive elements was still reaching these rostral segments. This is consistent with the finding that either complete medial or lateral lesions of the spinal cord rostral to the point of bending did not abolish entrainment; entrainment occurs locally and is distributed to other segments through the spinal coordinating system. However, entrainment was blocked by stripping away the most lateral part of the spinal cord in the region of bending, a procedure which undoubtedly destroys many of the local edge cells.

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258.5 **THE ROLE OF HIP MUSCLES DURING PAW-SHAKE RESPONSE.** **M.G. Hoy, R.F. Zernicke and J.L. Smith. Department of Kinesiology, UCLA, CA 90024.**

**During paw-shake response (PSR), characteristic muscle activity and joint oscillations occur at hip, knee, and ankle. Previously we reported on muscle and inertial moments during PSR for the knee and ankle joints (Hoy *et al.*, Neurosci. Abstr. 9:63, 1983), and now we have formulated three-segment rigid-body equations of motion and quantified hip joint kinetics during PSR steady-state cycles to understand the role of hip muscles and inertial moments in the control of PSR limb dynamics.**

**PSRs were elicited in four adult spinal cats by applying tape to the hindpaw, and limb motions were filmed (200 f/s). Raw data were smoothed with a cubic spline (Zernicke *et al.*, Res. Q. 47:9-19, 1976) and differentiated to obtain hindlimb kinematics. The thigh, leg, and paw were modeled as a planar, three-segment rigid-body system. Body segment parameters were obtained (Hoy & Zernicke, J. Biomech. 18:49-60, 1985), and kinetic equations of motion were formulated using Newtonian mechanics to yield hip, knee, and ankle joint moments. Gluteus medius (GM), biceps femoris (BF), and iliopsoas (IP) were chronically implanted with bipolar electrodes; EMG data were synchronized with kinematic and kinetic data.**

**The hip joint oscillated in-phase with the knee, and out-of-phase with the ankle during steady-state cycles. Both extensors (GM and BF) were active at the end of hip flexion and the start of extension, while IP was active during late hip extension and early flexion. The extensor muscle moment peaked (-250±13 mNm) subsequent to GM activity, and the flexor muscle moment peaked (501±125 mNm) near the end of IP bursts. Muscle moments counteracted large interactive moments, particularly those due to leg and paw angular acceleration; however, asymmetry existed in the pattern of inertial loading. Generally, during extension, segmental interactive moments peaked simultaneously but in opposite directions, reducing their net effect, while during flexion, peaks were desynchronized, requiring a prolonged flexor muscle moment to control thigh acceleration. The influence of thigh motion on knee and ankle joint dynamics was relatively small.**

**In contrast to the ankle, but similar to the knee, inertial moments were significant at the hip, and hip muscle moments counteracted interactive moments due to distal segment accelerations. Unlike knee and ankle dynamics, hip flexor muscle moments typically exceeded extensor muscle moments in duration and magnitude, suggesting a differential role for hip flexors and extensors in stabilizing hip oscillations and maintaining hindlimb position during PSR. Supported by NIH grant NS19864.**

258.6 DIFFERENTIAL CONTROL OF ANKLE AND KNEE MUSCLES WITH INTERSEGMENTAL PERTURBATIONS OF THE LIMB DURING PAW SHAKING. G. Koshland, E. Cox*, and J.L. Smith. Lab. Neuromotor Control, Dept. Kinesiology, UCLA, CA 90024.

Previous work in this laboratory (Hoy, et al. Neurosci. Abst. 1984) indicated differential control of ankle and knee muscle activity during the paw-shake response (PSR). Patterns of ankle muscle moment matched patterns of paw angular acceleration, while knee muscle moments counteracted inertial moments, particularly those generated by paw dynamics. These results led to our hypothesis that perturbation of limb dynamics would have greater effect on knee than on ankle muscle activity. We tested this hypothesis by assessing EMG patterns of PSRs elicited in hindlimbs perturbed by joint immobilization.

PSRs of 3 cats, spinalized at T-12, were studied under 4 or more conditions: 1 joint was immobilized (H, K, A), 2 joints (H-K, K-A), or three joints (H-K-A). PSRs were elicited by wrapping tape around the paw, and joints were immobilized with plaster casts. Cycle periods, burst durations, and intralimb synergies were assessed from EMG of representative flexor and extensor muscles.

With ankle joint immobilized (A, K-A) or with hip cast alone (H), reciprocal activity of ankle flexor (TA) and extensor (LG) muscles was preserved. Knee extensor (VL) and flexor (BF) activity, however, was variable with inconsistent onsets, low amplitude, and missing bursts. These results are consistent with our hypothesis.

Under other conditions (K, H-K, and H-K-A), however, reciprocal activity of flexor and extensor muscles at all joints was disrupted as all muscles cocontracted at a frequency of 8-10 Hz with burst durations of 40-60 ms. These results, not predicted by the hypothesis, suggest that ankle muscle activity may be influenced by the posture or dynamics of proximal segments.

It is striking, however, how similar the cocontraction pattern is to that described for shivering, another cyclic hindlimb behavior proposed to be centrally generated (Stuart, etal. Amer. J. Phys. Med. 45: 1966). This unexpected result suggests that shivering rather than the paw-shake synergy may have been elicited under some of our testing conditions. If common unit-burst generators (c.f., Grillner 1981) in the lumbosacral cord may be configured to control either behavior, it is possible that the state of the limb determines which is triggered. Supported by NIH grant NS 16984.

258.7 **INTRALIMB KINETICS DURING PAW SHAKES WITH DISRUPTED KNEE MOTION**

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**The paw-shake response (PSR) occurs in both normal and spinal cats, and in contrast to the strict alternation of the flexor/extensor muscle synergies of scratching and locomotion, the PSR has a mixed synergy with the vastus lateralis being coactive with tibialis anterior (Koshland & Smith, Neurosci. Abstr. 9:63, 1983). Kinetic analyses of the PSR (Hoy et al., J. Neurophysiol., Submitted) demonstrate that at the ankle joint, the muscle moment is highly correlated with the net joint moment, while at the knee the muscle moment is counterbalanced by inertial moments. Hence, it is predicted that the control of muscle moments is distinctly different at the knee and ankle joints during the PSR. In our current study, we altered the inertial characteristics of the cat hindpaw to investigate the mutability of muscle synergies and hindlimb intersegmental dynamics during the PSR.**

**PSRs were studied in adult spinal cats (T-12 transection) after weights (28 and 46 g) were attached to the proximal part of the paw. PSRs were elicited by wrapping tape around the paw, and EMG recordings of knee and ankle flexors and extensors were synchronized with kinematic data digitized from high speed cine film (200 f/s). The methods of Hoy and Zernicke (J. Biomech., 18:49-60, 1985) were used to determine paw and leg kinetics. EMG responses of the lateral gastrocnemius, tibialis anterior, vastus lateralis, and biceps femoris were recorded.**

**Weights had no significant influence on the average cycle periods, EMG onset latencies, burst durations, or on the number of paw shake cycles elicited. Angular kinematics of the weighted PSR movement demonstrated ankle and hip excursions similar to the unweighted shakes of the spinal cat, but knee movement, was disrupted as angular excursions were irregular and greatly reduced. In comparison to the unweighted PSR, the addition of mass to the paw resulted in a slight increase in the net and muscle moments at the ankle. At the knee, several factors contributed to a near-zero net moment, including a reduction of inertial terms except for an increased component due to paw angular acceleration that was effectively counterbalanced by the knee muscle moment.**

**Adding up to 46g to the cat's paw did not alter the neuromuscular patterns of the PSR; however, knee motions were markedly changed. Alterations in knee motions were not due to compensatory adjustments in the neural program, rather they emerged because of the interplay among the muscular and inertial moments that control the PSR. Supported by NIH grant NS19864.**

258.8 ACTIVITY OF RETICULAR FORMATION NEURONS DURING ACOUSTIC STARTLE IN BEHAVING CATS.

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In intact animals an intense acoustic stimulus elicits a startle reflex involving a phasic contraction of skeletal muscles in the whole body. Although previous lesion studies implicated the reticular formation (RF), particularly nucleus reticularis pontis caudalis (NRPC), as a critical structure mediating acoustic startle in mammals, no detailed data have been available on the activity of RF neurons during actual startle in these animals. In order to examine the role of RF neurons in startle, we have recorded RF unit activity during acoustic startle in behaving cats.

Adult female cats were implanted with mechanical microdrives containing movable bundles of 32 μm microwires into the medial ponto-medullary RF as well as EMG electrodes into three major neck muscles (splenius, biventer cervicis, complexus) bilaterally. Furthermore, three pairs of bipolar stimulating electrodes were chronically implanted into cervical cord (C2) and/or lumber cord (L1) in order to antidromically identify reticulospinal neurons. The activity of each isolated unit was observed systematically during various movements and sensory stimulation to determine its sensorimotor correlates. Unit and EMG responses to startle-inducing clicks or tone bursts (60-100 dB) were averaged by a computer.

The results may be summarized as follows. (1) Average neck EMG data indicated that the neck startle response had an onset latency of 6.5-8 ms and a duration of 5-15 ms. (2) Among the RF neurons which responded to startling stimuli, the response latency varied from 3.5 ms to more than 30 ms. Short-latency ( $\leq$ 7 ms) cells tended to cluster in NRPC. (3) Most of the responsive cells had motor correlates (neck flexion, facial movements, etc.) compatible or associated with startle. (4) Some of the antidromically identified reticulospinal cells had fast conduction velocities (about 100 m/s), short acoustic latencies (3.5-7 ms), and neck movement correlates.

In conclusion, these data support our hypothesis that certain fast-conducting reticulospinal cells normally related to neck movement act as relay neurons for the neck component of acoustic startle.

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258.9 THE ROLE OF THE LOWER BRAINSTEM IN THE CONTROL OF RHYTHMICAL JAW MOVEMENTS IN THE GUINEA PIG.

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It has been established that the basic rhythmical component of locomotion, respiration and mastication is produced by central neural networks (CPGs) which are strongly influenced by peripheral mechanisms. Based on anatomical and electrophysiological data from various laboratories the rhythmical jaw movements (RJMs) which can be induced by repetitive stimulation of the masticatory cortex in various animal species are produced by a masticatory CPG located in the brainstem. The precise location and neuronal elements which comprise this CPG have not been established. Furthermore, during RJMs the opener and closer muscles are alternately activated bilaterally. The question then arises as to whether there exists one CPG distributed between both halves of the brainstem or two functionally indepenent oscillators each residing in one half of the brainstem which are normally coupled during RJMs. The purpose of the present study was to determine in the anesthetized guinea pig with the technique of brainstem transections the elements of the lower brainstem which are essential for the occurrence of cortically induced RJMs and to what extent the integrity of both halves of the lower brainstem is critical for RJM production and bilateral activity in the jaw opener muscles.

Male albino guinea pigs were anesthetized and prepared for EMG recording from both left and right digastric muscles (jaw openers). Stimulating electrodes where inserted into both the right and left masticatory cortex to induce RJMs. The surface of the brainstem was exposed by suction ablation of the overlying cerebellum. Both midline transections and left hemisections of the lower brainstem starting at the obex were performed with a retractable fine wire knife. It was found that midline transections which extended from the obex to the rostral 1/3 of the Inferior Olivary Nucleus (I.O.) specifically abolished the ipsilateral digastric response to the repetitive cortical stimulus while leaving the rhythmical contralateral digastric response unaffected. Transections which extended between the rostral I.O and the Trigeminal motor nucleus (Mot V) completely abolished RJMs and both the ipsilateral and contralateral digastric response to the cortical stimulus. Left brainstem hemisections at the rostral I.O. and extending 2mm lateral to the midline were sufficient to abolish RJMs to stimulation of the right cortex. Under these conditions stimulation of the left cortex was capable of eliciting rhythmical activity only in the right digastric. Lesion which extended 1mm lateral to the midline which included the Nucleus Reticularis Gigantocellularis had minimal effects on RJMs. Hemisections rostral to Mot V abolished RJMs to stimulation of the left cortex but had relatively minor effects on RJMs induced by stimulation of the right cortex. Hemisections which spared the ventral quadrant rostral to Mot V had minimal effects on RJMs induced by stimulation of either left or right cortex.

These data demonstrate that the cortex of one side activates a CPG network residing in the contralateral brainstem which then coordinates bilateral digastric activity. It is suggested that the Parvocellular Reticular Formation between the I.O and Mot V contains either the CPG or output neurons of the CPG for RJMs in the guinea pig. This project was supported by NIDR-DE 06193.

258.10 LUMBAR AXIAL MUSCLE ACTIVITY IS CORRELATED WITH ELECTROENCEPHALOGRAM AROUSAL.

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Under urethane anesthesia (1.2-1.4 gm/kg), rat axial motor units exhibit variable levels of spontaneous activity. The EMG activity of lateral longissimus (LL) muscle can be increased or evoked in periods of silence by stimulation of the pudendal nerve (PN) (Cohen, Soc. Neurosci. Abstr., 316: 15, 1983). We have investigated the relationship between EEG arousal, and spontaneous and evoked EMG activity, in LL of adult female Sprague-Dawley rats. LL was exposed from vertebral levels L2 through L6. Multi-unit EMG recordings were obtained with a pair of longitudinally inserted 3 cm long teflon-coated 125 μm tungsten wires bared for 1 mm at 3 points along their length. The PN was stimulated with trains of 3 or 4 pulses repeated at 2-3 Hz. Individual pulses: 200 μs duration, at 667 Hz. Stimulus currents: 50-150 μA. The EEG was recorded between two screws placed in the frontal and parietal skull bones, ipsilaterally. In 5 rats, clear and abrupt changes in EEG and spontaneous activity were analyzed. 78% (215/277) of all changes in spontaneous activity of LL EMG (active to silent or vice versa) occurred within 20 s of changes in EEG state (synchronous to desynchronous or vice versa). 76% (215/284) of all changes in EEG state occurred within 20 s of changes in spontaneous activity. Where changes in spontaneous muscle activity were associated with changes in EEG, increased muscle activity always was accompanied by EEG desynchronization, and decreased muscle activity always was accompanied by EEG synchronization. In 11 rats, PN stimulation often desynchronized the EEG within 5 s, as did tail or ear pinch. In 87% (40/46) of the cases where the PN stimulus desynchronized EEG in the absence of spontaneous LL EMG, the stimulus also evoked EMG unit activity. In these cases, unit activity was never evoked prior to EEG desynchronization. In the 15 cases where EEG was not desynchronized by the stimulus, EMG units were not evoked. In 96% (53/55) of the cases where the stimulus was applied during the desynchronous phase of the EEG, unit activity was evoked, maintained or increased by PN stimulation. Stimulation of the nucleus gigantocellularis of the medullary reticular formation is known to desynchronize EEG (Moruzzi and Magoun, 1949), and has been shown to evoke lumbar axial muscle responses at brief latencies (Femano et al., Am. J. Physiol., 246: 1984). Individual gigantocellular neurons of rat project caudally to the spinal cord and rostrally to the intralaminar nuclei of the thalamus (Scheibel and Scheibel, in Reticular Formation of the Brain, 1958). Thus, reticular activation upon pudendal nerve stimulation could facilitate subsequent lumbar axial motoneuron responses to this stimulation. (Supported by PHS Grant HD 13795).

258.11 ELECTROPHYSIOLOGY OF MOTONEURONS IN THE SPINAL NUCLEUS OF THE BULBOCAVERNOSUS IN THE RAT. W.F. Collins, III, Dept. Obstetrics and Gynecology, Yale University School of Medicine, New Haven, CT 06510.

Striated penile muscles are important for the expression of penile reflexes in male rats (Sachs, J. Reprod. Fert. 66: 433-443, 1982). The motoneurons (MN) which innervate two of these muscles, the levator ani (LA) and the bulbocavernosus, are located in the spinal nucleus of the bulbocavernosus (SNB) (Breedlove and Arnold, Science 210: 564-566, 1980). The electrophysiology of SNB MNs was examined in adult (250-400g), anesthetized (Ketamine and Xylazine; 90 and 10 mg/Kg IM respectively plus maintenance doses) male Sprague-Dawley rats using in vivo intracellular recording techniques. The left LA muscle nerve (LLAN) and dorsal penile nerve (LDPN) were isolated and placed on bipolar platinum electrodes for stimulation. In 3 experiments, right and left ventral roots (L6) were also placed on stimulating electrodes. Glass micropipettes (3M KCl; 20-50MΩ) were advanced through the dorsal column (exposed by a laminectomy (T13-S1)) to penetrate SNB MNs. The present study includes SNB MNs identified antidromically following stimulation of the LLAN as well as following stimulation of ventral root L6. The antidromic action potentials were of normal configuration as compared to those of cat hindlimb MNs and were followed by a delayed depolarization (DD) and an afterhyperpolarization (AHP). Conduction velocity was determined (conduction distance/latency) for 30 MNs and ranged from 25 to 48 m/s (mean 33.2 +/- 0.24 (SE)). SNB MN rheobase was measured as the minimun depolarizing current (50 ms duration) required to generate an action potential and ranged from 1.4 to 9 nA (mean 3.8 +/- 0.18 (SE); n = 12). SNB MN input resistance was calculated from the slope of the I-V curve using hyperpolarizing current injection and ranged from 1.4 to 2.8 MΩ (n = 3). Synaptic input to SNB MNs was examined using stimulation of the LLAN and the LDPN. Reducing the stimulus intensity applied to the LLAN to below threshold for antidromic activation of the impaled MN revealed in several cases a graded monosynaptic (by latency measurements) EPSP. In most cases, LDPN stimulation resulted in a mixed, polysynaptic (by latency measurements) EPSP/IPSP. SNB MNs on the right side of the spinal cord (identified by stimulation of the right L6 ventral root) also responded to LDPN stimulation with synaptic potentials of similar configuration and latency. The presence of LDPN input to contralateral SNB MNs could result from contralateral projection of DPN afferents (Nunez et al., Neurosci. Abstr., 9, 1983) or contralaterally projecting SNB MN dendrites (Rose and Collins, Brain Res., in press).

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258.12 CATECHOLAMINE INNERVATION OF DENDRITE BUNDLES IN THE LUMBOSACRAL SPINAL CORD OF THE RAT. Anderson, W.J.[∓], D.L. Bellinger[+] and D.L. Felten[+]. Indiana Univ., Sch. of Med., Terre Haute Ctr. for Med. Ed., Terre Haute, IN[∓]; Dept. of Anat., Univ. of Rochester, Sch. of Med., Rochester, NY[+].

Anderson et. al. (1974) and Kerns and Peters (1974) described the morphology of 2 discrete dendrite bundles, one medial (MDB) and one lateral (LDB), in the 6th lumbar segment of the rat spinal cord. In this study, we examined these bundles and their catecholamine (CA) innervation using Golgi-Cox impregnation, fluorescence histochemistry for localization of CAs and acetylcholinesterase staining. Analysis of Golgi-Cox impregnated horizontal sections of MDB and LDB revealed fine varicose profiles that were similar to fluorescent CA fibers in size and appearance. These profiles descended in the anterior and lateral funiculi, then turned transversely, and entered the bundles at right angles. These fine varicose fibers then coursed horizontally, parallel to and sometimes in close apposition with, dendrites that formed the bundles.

CA histofluorescence of horizontal sections demonstrated a high density of varicosities within the MDB and LDB. These fluorescent fibers resembled the profiles seen in Golgi-Cox impregnated sections, as noted above. CA fibers entered the bundles perpendicular to the horizontally-oriented dendrites, and then turned rostrally or caudally to course adjacent to the dendrites. Varicosities formed pericellular rings around the motoneurons contributing to the bundles, and ran in linear arrays adjacent to the primary dendrites. The neuropil surrounding these dendrite bundles was innervated only sparsely by CA fibers, suggesting that the abundant innervation of the lumbosacral bundles is selective. A similar selective dense innervation of spinal cord dendrite bundles was noted in the cervical bundles of the rat (Bellinger et. al., Brain Res. Bulletin, in press). Alternate sections stained for specific acetylcholinesterase (AChE) demonstrated AChE-positive motoneurons and their dendrites within the bundles, and confirmed the apposition of CA varicosities with AChE-positive elements of the MDB and LDB.

The function of lumbosacral dendrite bundles remains unclear. Scheibel and Scheibel (1970) suggested that they may facilitate reciprocal activity of hindlimb muscles involved in stepping, walking and weightbearing. McKenna and Nadelhaft (1984) suggested that these bundles may subserve an excretory function in rats, as well as a possible sexual role in males. Dendrite bundles provide an ideal substrate for integration of synaptic inputs to interacting groups of homonymous and antagonist motoneurons. The presence of CA fibers adjacent to dendrites and perikarya within these bundles suggests that coordination of participating motoneurons may be regulated in part by supraspinal influences from the descending CA systems.

258.13 MORPHOLOGY OF SYNAPSES CONTACTING DISTAL DENDRITES OF NECK MUSCLE MOTONEURONS. P.K. Rose and M. Neuber*. Department of Physiology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

Much of the surface area available for synaptic contact on motoneurons is located on the dendritic tree. Indeed, a recent quantitative analysis of motoneurons innervating neck and shoulder muscles has shown that the surface areas of their dendritic trees constitute approximately 99% of the somato-dendritic surface area. Over 25% of the dendritic surface area is located more than 1,000 um from the cell body. These results emphasize the potential importance of the distal dendritic tree for the reception of synaptic inputs. Little is known however, about the morphology and density of synapses on the distal dendrites of motoneurons.

In the present experiments, neck muscle motoneurons were antidromically identified and intracellularly stained with HRP. The complete dendritic tree of one motoneuron was reconstructed from serial sections imbedded in Araldite. One group of rostrally projecting dendrites, 850 to 1,400 um from the cell body were subsequently examined at the electron microscopic level. HRP labelled dendrites were easily distinguished from the surrounding neuropil by the dense accumulation of the HRP reaction product in the cytoplasm. Each dendrite was identified by matching the location of HRP profiles seen in 0.3 um toluidene blue stained sections with the reconstructed dendritic tree.

A characteristic feature of distal dendrites is the relative paucity of synaptic contacts. Only 18% of the dendritic perimeter was contacted by boutons. The remaining membrane was enveloped by glia. Two types of synapses were identified. One contained spherical vesicles and the other contained pleomorphic or flattened vesicles. Many synapses were partially enveloped by the dendrite. Partial reconstructions using serial sections indicated that some of these synapses fitted into a trough along the dendritic shaft and thus, substantially increased the contact zone of the synapse. A preliminary examination of synapses on the cell body and proximal dendrites of two other motoneurons did not shown this specialization. Moreover, synaptic density on proximal dendrites was higher.

These studies indicate that distal dendrites of dorsal neck muscle motoneurons receive synaptic contacts. However, the density of synaptic contacts is low. If the activity of neighbouring synapses on distal dendrites and proximal dendrites is similiar, the loss of current along the dendritic path due to shunting would be less distally. Thus, the low density of synaptic contacts could significantly improve the efficacy of transmission from distal dendritic sites to the cell body. (Supported by the MRC of Canada.)

258.14 SYNAPTIC CONTACTS ON TWO POPULATIONS OF CERVICAL MOTORNEURONS IN A TURTLE, Pseudemys scripta. M.B.L. Yeow* & E.H. Peterson. Depts. of Zoology and Basic Science, Ohio University, Athens, OH 45701.

In the cryptodyran turtle, P. scripta, two populations of motorneurons (MNs) supply the cervical musculature: (1) ventral nucleus cells innervate neck muscles in a musculotopic pattern; and (2) medial nucleus cells are invariant in position (dorsomedial grey/white margin), extend dendrites into the contralateral spinal cord, and may mediate head and neck displacements in the mid-sagittal plane (Yeow and Peterson, '85a, '85b, J. Comp. Neurol., in press). Some but not all neck muscles are dually innervated by both populations of MNs. As a first step in examining the afferent circuitry of these MNs we have characterized the ultrastructure of synaptic boutons contacting ventral and medial nucleus MNs. Data were taken from spinal segment C3; each MN was sampled at 5um increments through the soma and horizontally oriented dendrites.

We have observed at least seven types of boutons contacting cervical MNs (Conradi, '69). Type S boutons bear clear, round vesicles (31-58nm) and asymmetric membrane densities. The mean length of apposition is 1.3um; variations in size, vesicle density, and ultrastructural characteristics suggest that there may be more than one morphological population of S bouton. Type F boutons bear pleiomorphic vesicles (up to 18x36nm) and thin symmetrical membrane densities ($\bar{X}$ appos. length=1.5um). M boutons resemble large S contacts ($\bar{X}$ appos. length=1.6um; vesicles 31-50um) and bear small P boutons on their convex surfaces. T boutons ($\bar{X}$ appos. length=1.7um) bear clear, round vesicles and postsynaptic dense bodies. C boutons are large ($\bar{X}$ appos. length=2um) with clear, round vesicles and a subsynaptic cistern. G boutons contain dense core vesicles (50-77nm); $\bar{X}$ appos. length= 1.3um).

The number of boutons per square um (synaptic density; Koziol & Tuckwell, '78, Br. Res. 150:617-624) was calculated for each bouton type observed in contact with MN soma, proximal, and distal dendrites. On both ventral and medial nucleus cells, the density of S and F type boutons is markedly higher than that of all other types, especially on dendrites. The density of F boutons is highest on proximal dendrites (.08 boutons/sq.um; soma-.04; distal dendrites-.03), while S bouton density increases with distance from the cell body (.03; proximal dendrites-.07; distal dendrites-.11). Ventral and medial nucleus cells differ in the relative densities of S and F type contacts: on all parts of the MN membrane, the density of S type contacts is higher on ventral nucleus cells whereas F type boutons are more dense on medial nucleus MNs. In addition, medially projecting dendrites of medial nucleus cells form chemical synapses with dendrites of unknown origin. These data suggest that ventral and medial nucleus MNs are under different patterns of synaptic drive; thus dually innervated cervical muscles may be controlled via two functionally distinct systems of motorneurons.

259.1 Effects of substance P (SP) and SP fragments on brain amine metabolism as determined by HPLC with electrochemical detection. M.E. Hall*, P. DeArmey*, M. Pelleymounter and J.M. Stewart. Dept. of Biochem., Univ. Colo. Health Sci. Ctr., Denver, and Dept. of Psychol., Univ. of Colo., Boulder, Colo.

Intraventricular (ICV) injections of substance P (SP) enhance spontaneous motor behaviors, such as hindlimb rearing and grooming, in rats (Rondeau, D., et al. Pharm. Biochem. Behav., 9: 769-775, 1978) and mice (Hall, M. and Stewart, J., Soc. Neurosci. Abstr., 10: 172, 1984). In rats, enhancement of rearing and grooming is thought to be mediated by SP's action on brain dopamine systems (Iversen, S., Substance P in the Nervous System, Pitman, 1982, 307-324). We have examined the effects, in mice, of ICV SP on levels of NE, DA, DOPAC, 5 HT and 5 HIAA in whole brain and in specific brain areas at various times after injection.

Brain tissue from peptide or vehicle-treated mice was homogenized in perchloric acid and a filtered supernatant subjected to reverse-phase HPLC on a C-18 column. Amines were quantified by a BAS electrochemical detector (+0.7V potential) and a Shimadzu integrator. Specific brain areas were removed by tissue punch from coronal sections (500 µm) of fresh frozen (-70° C) brain. Results (N=8-10/group) were analyzed by analysis of variance.

One microgram of SP (ICV) significantly alters motor behavior within 5 min. Five min. after such an injection, whole brain levels of NE and 5HT were significantly reduced. These effects were restricted to the mid- and hindbrain areas. SP also produced a significant increase in the DOPAC-to-DA ratio, suggesting an increase in DA metabolism, in the substantia nigra (SN). One hour after injection, DA was elevated and 5HT was reduced in the SN, while 5HT was also reduced in the caudate and the nucleus accumbens. A significant decrease in the 5HIAA-to-5HT ratio was observed in prefrontal cortex at 1 hr. At 24 hr. post injection, 5HT and DA were elevated in the caudate and the 5HIAA-to-5HT ratio was elevated in SN.

Amino (N-) and carboxy (C-) terminal fragments of SP have been shown to exert opposite effects on some motor behaviors (Hall & Stewart, ibid). The effects of N- and C-terminal fragments on brain amine levels were therefore examined. Opposite effects of these peptides on brain amine levels were also observed. For example, the N-terminal heptapeptide significantly increased, while the C-terminal hexapeptide significantly reduced, the 5HIAA-to-5HT ratio in the SN 1 hr. after injection.

These and other results will be presented, and discussed with regard to their possible causal relationship(s) to the effects of these peptides on motor behavior.

259.2 NEUROMEDIN N MIMICS THE ACTION OF NEUROTENSIN IN THE VENTRAL TEGMENTAL AREA BUT NOT IN THE NUCLEUS ACCUMBENS. P.W. Kalivas* and R. Richardson-Carlson* (SPON: R.C. Speth). Dept. of VCAPP, Washington State University, Pullman, WA 99164

Upon injection into the central nervous system of rodents neurotensin (NT) has been shown to produce many "neuroleptic-like" effects, including blockade of the motor stimulant effect of intra-accumbens injection with dopamine (DA). Paradoxically, injection with NT into the ventral tegmental area (VTA) produces an increase in mesolimbic DA turnover and corresponding behavioral hyperactivity. Neuromedin N (NN) was recently characterized in porcine spinal cord as a hexapeptide having a four amino acid homology with the C-terminus of NT. Since the six amino acid fragment of NT that contains the C-terminus demonstrates equivalent biological activity to the parent compound, we examined whether NN has biological activity in some of the centrally-mediated effects of NT.

Male S.D. rats were implanted with chronic bilateral injection cannulae over the VTA or nucleus accumbens. One week after surgery, either NN, NT or sterile saline vehicle was microinjected into the VTA at various doses in 0.2 µl/side over 60 sec. After injection the rats were placed in a photocell apparatus and motor activity monitored for 120 min. NN produced a significant increase in motor activity with a threshold dose between 0.03 and 0.1 nmole/side. The threshold dose for NT was between 0.33 and 1.0 nmole/side. To determine if NN was also more potent at increasing mesolimbic DA turnover, rats were given intra-VTA injection with saline or 0.33 nmole/side of either NN or NT, decapitated 60 min later, and DA and its metabolites were measured in the nucleus accumbens using HPLC-EC. At this dose, both NN and NT produced an equivalent increase in the levels of DA metabolites. In a second study, either NT, NM, NM + DA, NT + DA or saline was injected into the nucleus accumbens in a volume of 0.5 µl/side over 60 sec. Following injection, the rats were placed in a photocell apparatus. At a dose of 1.0 nmole/side, NT completely abolished the effect of DA (30 µg/side), while the equivalent dose of NN was totally ineffective.

These data show that NN produces a similar effect to NT in the VTA, but does not share the "neuroleptic-like" qualities of NT in the nucleus accumbens. While these data are suggestive of separate receptors for NN and NT in the brain, we are currently characterizing the NN receptor to directly evaluate this hypothesis.

259.3 DISSOCIATION OF TOLERANCE AND DEPENDEENCE TO MORPHINE: A POSSIBLE ROLE FOR CHOLECYSTOKININ. P.Sacerdote*, P.Mantegazza*; L.C.Rovati*, and A.E.Panerai. Dept. Pharmacology, Chemotherapy and Medical Toxicology, School of Medicine, Univ. of Milano, 20129 Milano, Italy. (Spon. L.M.Vicentini)

Proglumide, a cholecystokinin receptor antagonist, potentiates morphine induced analgesia, and reverses tolerance to morphine experimentally induced in the rat by means of short term (14 hrs/6 days) parenteral treatment with the opiate.

In the present study we looked at the effects of the concomitant administration of morphine and proglumide on the development of tolerance during long term administration of the opiate, similarly to what happens in clinical practice.

Moreover, we looked at the effects of proglumide on the development of dependence.

Drugs were administered either orally dissolved in drinking water, parenterally or orally.

Under all experimental conditions the concomitant administration of morphine and proglumide inhibited the development of tolerance to the analgesic effect of the opiate.

However, proglumide did not prevent the withdrawal syndrome induced by the administration of naloxone as evaluated by either graded (e.g. wet dog shakes, self stimulation, writhing), quantal (e.g. diarrhea, teeth chattering, irritability to touch) signs, and body weight decrease.

Our data induce to confirm the hypotheses that a cholecystokinin like peptide might play a role in the development of tolerance to morphine and that proglumide might induce the effects we showed through an inhibition of cholecystokinin.

In order to further evaluate these possibilities, studies are in progress with other substances that show in in vivo and in vit studies a cholecystokinin antagonistic activity 500/600 folds that of proglumide.

Acknowledgements

We thank Rotta labs., Monza, Italy for proglumide and otheer anticholecystokinin compounds.

259.4 EFFECT OF PENTYLENETETRAZOL (PTZ) KINDLED SEIZURES ON TRH LEVELS IN SPECIFIC AREAS OF THE RAT BRAIN. M.J. Kubek and S.L. Morzorati. The Regenstrief Institute and Departments of Anatomy and Psychiatry, Indiana University School of Medicine, Indianapolis, IN 46223.

Thyrotropin-releasing hormone (TRH) has been demonstrated to exist in extrahypothalamic sites where it may function as a neurotransmitter and/or neuromodulator. Recent studies in our laboratory have shown TRH to be elevated in specific limbic and cortical structures from 2 to 12 days after generalized seizures were induced by either electrical kindling (Neurosci. Abstr., 9:485, 1983) or electroconvulsive shock (ECS) (Life Sci., 36:315-320, 1985 and this meeting). The pattern of TRH elevation seen following kindling is similar to that observed with ECS. In order to extend these studies and to rule out a possible direct electrical effect on TRH, the present investigation examined the effect of chemical kindling on the TRH response. Male Wistar rats (250-270g) were injected once daily with a subconvulsant dose of PTZ (20mg/kg; ip). An animal was considered fully kindled after having stage-5 seizures on 3 consecutive days. This process took 20 +/- 4 days. A control group was treated identically with saline. After kindling, controls and experimentals were injected with either saline or PTZ (20 mg/kg) once a month for 6 months. The last treatment was given 48hrs prior to decapitation and the kindled subjects had stage-5 seizures within 30 min. Brains were removed, quickly dissected into 8 regions and frozen on dry ice. Tissues were extracted with HAc and TRH was quantitated by a specific and sensitive radioimmunoassay. TRH content was expressed as pg/mg wet weight (mean +/- SEM). Of the regions thus far examined, chemical kindling induced a marked increase in TRH in the Amygdala (40.85 +/- 2.2 vs 66.21 +/- 6.6; $p < 0.005$) and a 6-fold elevation in the Pyriform Ctx. (6.52 +/- 0.93 vs 30.36 +/- 2.79; $p<0.001$). These preliminary results are consistent with those seen with electrical kindling and ECS and thus rule out a possible direct electrical effect on the TRH response to seizures. These data further support the concept that endogenous TRH may be involved in the pathophysiology of epilepsy. Supported by the Veterans Administration Research Service and NIADDK AM-28260 (M.J.K.).

259.5 **EFFECT OF INHIBITORS OF MEMBRANE-BOUND METALLOENDOPEPTIDASE "ENKEPHALINASE" ON NEURONAL ACTIVITY IN THE SUBSTANTIA NIGRA OF THE RAT.** M.J.Bier*, J.Goldfarb, M Orlowski and S.Wilk.Depts.of Pharmacology and The Neurobiology Program*,Mt.Sinai School of Medicine, New York, NY 10029.

In rat brain the highest concentrations of substance P (SP) and enkephalins (ENK) are found respectively in the caudato-putamen (CP) and globus pallidus. A major target of this peptidergic outflow is the substantia nigra (SN).The metallo-endopeptidase (EC 3.4.24.11), has its highest activity in the SN and CP and was shown to be identical with the enzyme commonly refered to as "enkephalinase" (Almenoff,J. et.al.BBRC 120: 206,1981).The metalloendopeptidase degrades both ENK and SP and has a higher affinity for SP than for ENK. Inhibition of EC 3.4.-24.11 terminates the hydrolysis of SP in a preparation of striatal synaptosomal membranes (Fulcher,I.et.al. Biochem.J.203:519, 1982). Several specific inhibitors of the enzyme have been synthesized including N-(1-(R,S)-carboxy-3-phenylpropyl)-Phe-pAB (PP) and N-(1-(R,S)-carboxy-2-phenylethyl)-Phe-pAB (PE)(Almenoff,J.and Orlowski,M.Biochem.,22:590,1983). We used these inhibitors in vivo to explore their effects on the electrical activity of cells in the SN.

Male Sprague-Dawley rats, anesthetized with urethane, were secured stereotaxically for recording. Electrical stimulation of the left CP at, or rostral to, the anterior commissure was used to evoke responses in the SN. Extracellular recordings were made ipsilateral to the stimulus in both the SN compacta (SNc) and the SN reticulata (SNr). Inhibitors were infused into the right lateral ventricle at a rate of 1uM in 10min.

Responses evoked in the SN were either excitatory, mixed excitatory/inhibitory, or purely inhibitory. The cells which showed an excitatory component (+) responded to the inhibitor treatment with an increase in their spontaneous firing frequency 15-50% above baseline. Those cells whose evoked responses were inhibitory (-) evinced no response to the inhibitors. Similar results were obtained with PE($Ki=3.9x10^{-7}$) and PP($Ki=3.7x10^{-7}$). Both SNc and SNr cells responded to the drugs. Maximum firing frequency was observed 15-20 min after termination of the infusion. The inhibitors did not induce any unusual patterning in the SN units.

Inhibitors of the enzyme prolyl-endopeptidase (Wilk,S.Life Sci. 33:2149,1983),a cytosolic enzyme which also degrades SP in vitro, did not affect the firing of (+) or (-) cells in any reproducible manner.

These results are consistant with the idea that the membrane bound metalloendopeptidase is involved in regulating the activity of an excitatory peptide in the SN.

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259.6 CORTICOTROPIN RELEASING FACTOR (CRF): CENTRAL NERVOUS SYSTEM (CNS) SITE OF ACTION. M.R. Brown. Clayton Foundation Laboratories for Peptide Biology, The Salk Institute, La Jolla, CA 92037

CRF administered to rats by either intraventricular or intracisternal routes elevates plasma concentrations of epinephrine and norepinephrine and increases mean arterial pressure and heart rate. Based on the existing knowledge of CRF-like peptide and receptor distribution in the brain and of the CNS areas suspected to be involved in the regulation of the autonomic nervous system, studies have been performed to identify the CNS site(s) of action of CRF to increase plasma concentrations of epinephrine and norepinephrine. Experiments were performed in conscious rats equipped with right atrial and brain ventricular and parenchymal cannulae (28 gauge). Peptide or vehicle was administered in 100 nl volumes over 30 seconds. Cannulae placements were determined histologically. A dose response to CRF administered into the third ventricle demonstrated that 200 pmoles of peptide elicited a significant submaximal elevation of plasma concentrations of epinephrine and norepinephrine. CRF (200 pmoles) administered into the lateral, third and fourth ventricles or cisterna magnum produced similar elevations of plasma catecholamines. CRF (200 pmoles) was microinjected into more than 100 different brain areas; no correlation was found between the magnitude of the elevation of plasma catecholamines following CRF injection and the reported distribution density of CRF-like peptides and receptors in these areas. Significant elevations of plasma concentrations of catecholamines were observed in animals with injections of CRF into brain areas beginning rostrally at the anterior hypothalamus extending caudally into the brain stem; no sites within this zone appeared more responsive than another to CRF administration. The magnitude of elevation of plasma catecholamines following CRF injection into various brain areas did not depend on the proximity to surrounding ventricular structures.

The results of this study support the concept that CRF acts through brain receptors ranging through a long rostral-caudal extent. Although brain areas were identified to be unresponsive to CRF e.g. striate cortex, caudate-putamen, olfactory bulb and peoptic and septal areas, no apparent single site of action of CRF could be determined.

259.7 **5β-METABOLITES OF PROGESTERONE STIMULATE LHRH RELEASE FROM THE RAT HYPOTHALAMUS SUPERFUSED IN VITRO.** K.Kim, O.Park* and V.D. Ramirez. Department of Physiology and Biophysics, University of Illinois, Urbana, IL 61801.

Previous works using prepuberal ovariectomized, estradiol-primed (OVX+E) rats have shown that progesterone can stimulate LHRH release from in vitro hypothalamic preparations.

The present study examined the effect of 5β-metabolites of progesterone on in vitro LHRH release from hypothalamic fragments from OVX+E adult rats. The hypothalamic fragments including preoptic area, suprachiasmatic nucleus, anterior hypothalamic area and mediobasal hypothalamus (POA-SCN-MBH) were placed in a superfusion chamber (one unit/chamber) and superfused with modified Krebs-Ringer phosphate medium (pH 7.40). After 1-hr equilibration period, perfusate samples were collected on ice at 10 minute intervals for 3 to 3.5 hrs. Progesterone, pregnanolone and epipregnanolone were infused in either an intermittent (10-min on, 20-min off) or continuous mode. LHRH concentrations were determined by a sensitive and specific radioimmunoassay. Intermittent infusion of progesterone (10 ng/ml but not 1 ng/ml) stimulated in vitro LHRH release. Interestingly, much lower doses of pregnanolone (0.01 mg/ml) also stimulated in vitro LHRH release when infused in an intermittent mode. This release was characterized by two distinct peaks with the initial peak triggered approximately one hr following infusion. Mean LHRH release (X±SE, n=5) induced by an intermittent infusion of pregnanolone was significantly ($<0.05$) higher than its preinfusion values and that of control group (1.56±.21 vs .80±.04 and .87±.07 pg/10 min). In contrast, continuous infusion of pregnanolone did not evoke in vitro LHRH release, but rather decreased it. Similar results were observed when epipregnanolone, another 5β-metabolite, was infused in an intermittent mode.

These data clearly demonstrated that intermittent, but not continuous infusion of 5β-metabolites of progesterone are highly effective in stimulating in vitro LHRH release from hypothalamic fragments of OVX+E primed adult rats, within an order of potency approximately 1000-fold greater than progesterone. These results suggest that 5β-metabolites of progesterone are important signals in controlling the neural LHRH apparatus.

259.8 CENTRAL THYROTROPIN-RELEASING HORMONE INDUCES SYSTEMIC HYPOGLYCEMIA IN MICE. S. Amir, A.I. Rivkind* and M. Harel*. Dept. of Isotope Research, Weizmann Institute of Science, 76100 Rehovot, Israel.

Thyrotropin-releasing hormone (TRH, pGlo-His-Pro-$NH_2$), injected centrally, has profound and varied autonomic effects which are independent of its endocrine functions. For example, central TRH increases blood pressure, heart rate and respiratory rate, stimulates gastric acid secretion and enhances gastro-intestinal motor activity. In rats, central TRH also elicits systemic hyperglycemia which is mediated by release of adrenal medullary catecholamines and pancreatic glucagon. In the present study, intracerebroventricular (ICV) microinjection of TRH (1-10 µg) in mice produced powerful long lasting (> 3h) hypoglycemia as well as antagonized the rise in plasma glucose produced by systemic injection of epinephrine (50 µg), glucagon (50 µg), 2-deoxyglucose (500 mg/kg), apomorphine (5 mg/kg), clonidine (0.25 mg/kg) or morphine (5 mg/kg). The insulin-like hypoglycemic effect of central TRH could be reproduced by ICV injection of acid TRH (pGlu-His-Pro-OH, 10 µg), a metabolite of TRH lacking hypophysiotropic influences, as well as by the neuroactive TRH analog, γ-butyrolactone-γ-carbonyl-His-Pro-$NH_2$ (DN-1417, 10 µg), but not by another TRH metabolite, cyclo [His-Pro] (1-10 µg) or two structurally related peptides, pGlu-His-Gly-$NH_2$ and pGlu-His-Trp (1-10 µg). The centrally-mediated hypoglycemic effect of TRH was blocked by pretreatment with the muscarinic cholinergic antagonist atropine methyl bromide (1 mg/kg S.C.), which selectively inhibits peripheral parasympathetic activity or by the administration of the cytotoxic compound alloxan (100 mg/kg), which destroys insulin producing beta cells in the pancreas. Disruption of sympathetic effector mechanisms by pretreatment with the ganglionic blocker chlorisondamine chloride (10 mg/kg) or the adrenergic receptor antagonists phentolamine and propranolol (10 mg/kg) had no effect on the hypoglycemic response to central TRH. The results indicate that in mice TRH acts in the brain to elicit a cholinergic (parasympathetic)-mediated insulin-dependent glucoregulatory response which can diminish the normal plasma glucose concentrations as well as antagonize the hyperglycemia produced by compounds that stimulate endogenous glucose production. The results support the concept that TRH in the brain may play a role in the central regulation of glucose homeostasis.

259.9 INTRACISTERNALLY ADMINISTERED OXYTOCIN PRODUCES HYPERTHERMIA AND ANALGESIA IN MICE. G.A. Mason*, J.D. Caldwell*, O.H. Hatley*, D.A. Stanley*, A.J. Prange, Jr. and C.A. Pedersen. (SPON: M.A. Lipton) Biological Sciences Research Center, Department of Psychiatry and the Neurobiology Curriculum, University of North Carolina at Chapel Hill, NC 27514

Both elevated colonic temperatures and increased analgesic responses were observed in adult male Swiss Webster mice 30 min after intracisternal (I.C.) administration of oxytocin (OXY).

For thermoregulatory studies, Mice were acclimated for one hr in individual Plexiglas cages without bedding at an ambient temperature of 22°C. They were injected with OXY and/or equimolar amounts of neurotensin (NT), bombesin (BOM), arginine vasopressin (AVP) or the 0.9% saline vehicle. Colonic temperatures were recorded at 0, 30, 60, 90 and 120 min postinjection. A dose of 1 or 4 μg of OXY significantly ($p < 0.01$) increased colonic temperature after 30 min (Dunnett's T versus vehicle = 3.7 and 4.3 for 1 and 4 μg, respectively, d.f. = 12). Simultaneous I.C. administration of 2 μg OXY also significantly ($p < 0.05$) attenuated the hypothermia produced at 30 or 60 min by equimolar amounts of NT or BOM (Tukey's Test for Multiple Comparisons). The temperature effects of OXY (4 μg) and an equimolar dose of AVP (which also produced a hyperthermic response) were not additive when the two peptides were given simultaneously. An intraperitoneal (I.P.) injection of indomethacin (5 mg/kg) failed to block the hyperthermic response of OXY (1 μg), thereby indicating that the effect was not dependent on release of prostaglandins.

Centrally administered OXY also produced a positive response in the hot plate analgesia test. Mice were placed on an enclosed copper plate set at a temperature of 52.5 ± 0.5°C, and the time elapsed before the animals jumped or licked their paws was recorded. Animals were removed after such responses or after 30 sec if they had not responded. Each mouse was tested just prior to peptide administration and at 30, 60, 90 and 120 min postinjection. OXY (1 or 4 μg) produced a significant ($p < 0.05$) analgesic response at 30 min (Tukey's Test for Multiple Comparisons). This response was not blocked by naloxone (5 mg/kg) administered I.P. 30 min before I.C. OXY.

259.10 Thymosin stimulates hormone secretion from AtT20 mouse corticotropic tumor cells. J.M.Farah, Jr., J.F.Bishop, J.P. McGillis, N.R. Hall, A.L. Goldstein* and T.L.O'Donohue. Experimental Therapeutics Branch, NINCDS, NIH Bethesda, MD 20205 and Department of Biochemistry, George Washington University School of Medicine, Washington, DC 20037

A number of neuroendocrine regulators are known to govern the secretion of pro-opiomelanocortin (POMC) peptides (e.g., ACTH and β-endorphin) from corticotrophs of the anterior pituitary. Recently, thymosin fraction 5 (TSN-5), a family of thymic peptides, was found to activate the pituitary-adrenocortical axis in primates as determined by its ability to elevate blood levels of immunoreactive ACTH, β-endorphin and cortisol (Healy et al, 1983, Science 222:1353). In order to determine if TSN-5's ability to increase POMC secretion is mediated through direct stimulation of corticotrophs, we studied the effects of TSN-5 on secretion of immunoreactive β-endorphin (iβ-E) by the AtT-20 mouse corticotrophic tumor.

AtT20/D16-16 cells were grown in Dulbecco's Modified Eagle's Medium fortified with fetal bovine serum and antibiotics. Levels of iβ-E in release medium was measured by radioimmunoassay using a rabbit antiserum (C-55) provided by Dr. Gregory Mueller which detects β-E and its immediate precursor, β-lipotropin, equally well. Release of iβ-E was increased relative to control within 15 min after exposing AtT-20 cells to 200 μg/ml of TSN-5 (4.4 ± 0.5 vs 3.1 ± 0.1 ng/ml, mean ± SEM, n=6) and elevated release was observed up to 4 hrs after treating the cells with TSN-5. Under present conditions, doses of TSN-5 between 0.06 and 60 μg/ml had no effect on basal secretion of iβ-E nor on release evoked by moderate (1 nM) to high doses (0.1 μM) of rat corticotropin releasing factor (CRF). TSN-5 (600 μg/ml) and CRF (0.1 μM) separately increased release of iβ-E to 148% and 221%, respectively, of control levels (16.5 ± 1.7 ng/ml in 4 hrs, n=7) and together evoked an additive increase in hormone release. The additive effects of doses of TSN-5 and CRF which alone cause near maximal secretion of hormone indicate that actions of TSN-5 may occur by mechanisms independent of cyclic adenosine monophosphate, the second messenger which mediates CRF stimulation of corticotrophic cells. The present results are consistent with recent findings that TSN-5 stimulates ACTH release in primary cultures of rodent anterior pituitary (McGillis et al, 1985, Fed Proc 44: 3428A) and, together, suggest that a thymic derivative may join the list of substances with releasing activity on corticotrophs. The identity of the active peptide(s) in TSN-5 and its mechanism for stimulating POMC secretion in the mouse corticotrophic tumor is presently under investigation.

259.11 CENTRAL EFFECTS OF AN ANTISERUM AND AN ANTAGONIST TO SUBSTANCE P ON GONADOTROPIN, AND PROLACTIN SECRETION IN THE RAT. Carl W. Skelley, W. Les Dees and Gerald P. Kozlowski. Department of Physiology, University of Texas Health Science Center at Dallas, Dallas, Tx 75235.

Intraventricular administration of substance P (SP) to laboratory animals has been shown to cause an increase (Vijayan and McCann, Endo., 105:64, 1979) or a decrease (Eckstein et al., Neuroendo., 31:338, 1980) in serum LH with no change in serum FSH levels. Additionally, reports have been conflicting as to whether an intraventricular injection of SP causes an increase (Vijayan and McCann, Endo., 105:64, 1979) or has no effect (Chihara et al., Endo., 102:281, 1978; Maeda and Frohman, Endo., 103:1903, 1978) on PRL secretion. SP-analogues having antagonistic properties, as well as antisera to SP have been widely used in the evaluation of possible roles of this peptide in physiology. If indeed these substances are truly antagonists, then their experimental use could provide additional information to aid in determining whether or not SP plays a central physiological role with regard to gonadotropin and PRL secretion. In this regard, we have studied the central effects of both of these substances on gonadotropin and prolactin (PRL) secretion using castrated male rats. A lateral ventricular injection of an analogue to SP having antagonistic properties caused significantly lower serum LH levels without altering serum FSH and PRL when compared to saline-injected controls. Similarly, a lateral ventricular injection of anti-SP also resulted in significantly lower levels of LH when compared to control animals injected with normal rabbit serum. Additionally, no changes were observed in the levels of serum FSH and PRL as a result of the anti-SP injection. These results support the hypothesis that SP may have a central stimulatory action on LH secretion, but not on FSH and PRL secretion.

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259.12 MULTIPLE FACTORS REGULATE THE RELEASE OF ATRIAL NATRIURETIC PEPTIDES FROM THE CARDIAC ATRIA. N. Zamir,* R.L. Eskay,* M. Haass,* J.R. Dave,* H. Keiser,* and Z. Zukowska-Grojec.* (SPON: J.W. Bigbee) Lab. of Clin. Science, NIMH; Lab. of Clin. Studies, DIR., NIAAA; Hypertension-Endocrine Branch, NHLBI; NINCDS; N.I.H., Bethesda, Md. 20205. U.S.A.

Evidence suggests that the mammalian atrium is an endocrine organ that may be involved in the control of blood pressure and extracellular fluid volume. A group of peptides, which appear to be associated with atrial-specific secretory granules, have potent natriuretic, diuretic and smooth muscle relaxant activities. These atrial peptides are termed atrial natriuretic peptides (ANPs), and have been shown to be derived from a common precursor. The results of several studies suggest a hormonal role for the ANPs. Utilizing an antiserum which recognizes rat alpha-ANP (3-28) alpha-ANP(5-28), alpha-ANP(5-27) and alpha-ANP(5-25) on an equimolar basis, we have determined immunoreactive (IR) levels of ANPs in rat plasma. Blood samples were obtained from conscious, unrestrained rats with indwelling arterial catheters and aliquots of plasma were extracted for ANPs with approximately an 80% recovery. Characterization of IR-ANPs present in extracted plasma by high performance liquid chromatography revealed two major peaks. The elution profile of these peaks suggests that they correspond to alpha-ANP (3-28) and alpha-ANP (5-28). Basal plasma concentrations of IR-ANPs in male Sprague-Dawley rats were 125 ± 12 pg/ml (Mean ± SEM, N=6). Acute volume load (intravenous infusion of 20 ml of 5% glucose/kg body weight over 1 min.) increased IR-ANPs 5-6 fold in plasma within 2 minutes. Similarly, infusion of 1 ml of hypertonic saline (3mEq NaCl) over 2 min. increased plasma levels of IR-ANPs 5-6 fold within 2 minutes. Ganglionic blockade with chlorisondamine, (10 mg/kg, IV) resulted in a 2-3 fold increase in plasma ANPs within 15 minutes. Our findings indicate that distension of the atria and osmotic stimulus enhance the release of ANPs and that the neural integrity of the heart is important for ANPs release from the cardiac atrium.

259.13 DECREASED CENTRAL ANGIOTENSINERGIC AMINOPEPTIDASE ACTIVITY IN SPONTANEOUSLY HYPERTENSIVE RATS. J.W. Wright, T. M. Woods*, D.L. Rochelle*, S. J. King* and J.W. Harding. Departments of Psychology and Veterinary and Comparative Anatomy, Pharmacology and Physiology, Washington State University, Pullman, WA 99164-4830.

Intracerebroventricular injections of angiotensin antagonists are differentially effective at lowering blood pressure in spontaneously hypertensive (SH) rats as compared with Wistar-Kyoto (WKY) control rats (Ganten et al., 1975; Phillips, 1980, 1983; Phillips et al., 1975; Sweet et al., 1977). And stroke prone SH rats reveal increased electrophysiological sensitivity to centrally applied angiotensin II (AII; Schnelling and Felix, 1983), and a greatly prolonged response of subfornical organ neurons in SH rats to microiontophoretically applied AII (Felix, personal communication).

Recent studies from our laboratory have also indicated enhanced and prolonged pressor responsiveness of SH rats to intracerebroventricularly applied AII and angiotensin III (AIII), consistent with the notion that SH rats are more sensitive to intracerebroventricular angiotensins. Additional rats were prepared with intracerebroventricular cannulas and were injected with [$^{125}$I]AII or [$^{125}$I]AIII and their brains were microwave irradiated to stop peptidase activity at intervals ranging from 5 through 60 seconds. The SH rats were found to have longer angiotensin survival times compared with WKY and Sprague-Dawley control animals as determined by High Performance Liquid Chromatography analyses suggesting an impairment in the ability of ventricular membrane-bound peptidases to metabolize angiotensin. Membrane-bound peptidases were implicated because incubation of these ligands in freshly collected cerebrospinal fluid from SH, WKY and Sprague-Dawley rats resulted in nondifferential delayed metabolism with approximately 91 and 52% of the [$^{125}$I]AII and [$^{125}$I]AIII respectively, remaining intact after 90 minutes at 37° C. In addition the supersensitivity of intracerebroventricularly injected angiotensins was observed to increase following pretreatment with the nonspecific aminopeptidase inhibitor, bestatin, in members of all three strains.

These results support the hypothesis that SH rats may have a dysfunction of angiotensin receptor associated peptidases which is evidenced as an impairment in angiotensin signal termination, thus resulting in sustained elevations in blood pressure.

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259.14 PRESSOR EFFECTS OF PERIPHERALLY INFUSED ANGIOTENSIN II AND III IN THE CONSCIOUS RABBIT. M. J. Sullivan, R. H. Abhold, J. W. Harding and J. W. Wright. Departments of Psychology and Veterinary Comparative Anatomy, Pharmacology, and Physiology, Washington State University, Pullman, WA 99164.

Binding studies utilizing [$^{125}$I] AII and AIII reveal a distribution of receptors in the rabbit brain similar to that seen in primates (Wright, Sullivan, Peterson and Harding, in prep). The majority of sites bind [$^{125}$I] AIII; relatively few AII sites are seen. These observations suggested that the rabbit might be an appropriate primate model as opposed to the rat which has proportionately more AII binding in the brain.

In the present study, mature male New Zealand rabbits were adapted to a restrainer daily for two weeks and were next accustomed to the insertion of an infusion needle in the marginal ear vein and a second needle in the central ear artery of the opposite ear for monitering blood pressure. Comparisons were made of pressor responses to 5 min of peripherally infused doses of AII and AIII. Doses of 1 and 10 pmol/kg/min AII and AIII were equipotent and had minimal effect on mean arterial blood pressure (MAP). At doses of 100 pmol/kg/min, AIII yielded 52% the pressor activity of AII. Radioimmunoassay (RIA) of angiotensins levels in blood samples taken during infusion of 100 pmol/kg/min AIII indicated 53% as much angiotensin as during infusion of an equimolar dose of AII. The antibody used in the RIA was cross reactive between AII and AIII and values represent the combined levels of the two peptides. During infusions of 500 pmol/kg/min, AIII yielded 64% the pressor response and 67% the plasma angiotensin level seen during infusion of AII. The half-life of AII is nine seconds as is the half-life of AIII. Since AII is converted to AIII, it has an effective half-life of 18 seconds, while aminopeptidases cleave AIII to an inactive form. Therefore during an infusion of AII, more ligand is available to interact with receptors. Our results suggest that AIII is at least as potent as AII with regard to pressor responses.

| Infusant | | Maximum Pressor Response from Baseline (mm Hg) | Plasma angiotensin (pg/ml) |
|---|---|---|---|
| 100 pmol/kg/min | AII | 24.6 | 117.7 |
| | AIII | 12.9 | 62.6 |
| 500 pmol/kg/min | AII | 44.7 | 287.5 |
| | AIII | 28.4 | 191.3 |

259.15 CARDIOVASCULAR EFFECTS OF CALCITONIN GENE-RELATED PEPTIDE IN THE PITHED RAT: COMPARISON WITH SUBSTANCE P. M. Haass*, I.J. Kopin, D.M. Jacobowitz, Z. Zukowska-Grojec* and G. Skofitsch*. Sec. of Pharmacology, NINCDS; Lab. of Clinical Science, NIMH, NIH, Bethesda, MD 20205.

Alternative processing of the primary transcript of the rat calcitonin gene has resulted in a novel 37 amino acid peptide, calcitonin-gene related peptide (CGRP). Immunohistochemical studies have shown that CGRP is located in central and peripheral nerve endings especially of primary sensory neurons. CGRP-staining was also observed in thin fibers associated with the smooth muscle of blood vessels and with the heart. Recently we found that CGRP coexists with substance P (SP) in cell bodies of sensory neurons of the trigeminal and the dorsal root ganglia. Since CGRP affects cardiovascular function in conscious rats, we compared the vasodepressor and the plasma extravasating activities of CGRP with those of SP in pithed vagotomized ratsto evaluate direct cardiac and vascular effects without interference of centrally mediated circulatory reflexes. Systemic administration of CGRP (0.1-10.0 μg/kg) evoked a dose-dependent, long-lasting vasodilation with a peak effect after 1 minute. High doses of CGRP also caused a tachycardiac response which was not blocked by pretreatment with the beta-adrenoceptor antagonist, propranolol (2 mg/kg, i.v.), suggesting a direct positive chronotropic action of CGRP. No significant change in heart rate was observed following SP administration (0.1-10.0 μg/kg, i.v.). For any given dose duration and degree of the hypertensive effect of SP was much smaller than that of CGRP. After either CGRP or SP, there was a more pronounced decrease in mean arterial pressure (MAP) after elevation of the basal blood pressure levels by constant infusion of phenylephrine (3 μg/kg/min.,i.v.):

| | (n) | dose (μg/kg) | MAP ± SEM (mm Hg) C | 1 min | 3 min |
|---|---|---|---|---|---|
| CGRP | (7) | 0.3 | 103.1 ± 3.8 | 92.9 ± 2.6* | 100.6 ± 2.7 |
| | | 1.0 | 104.9 ± 3.4 | 76.1 ± 3.5** | 90.6 ± 3.4* |
| | | 3.0 | 109.0 ± 2.4 | 71.0 ± 4.0*** | 83.7 ± 4.8** |
| SP | (7) | 0.3 | 111.1 ± 6.1 | 111.4 ± 5.0 | 111.7 ± 4.8 |
| | | 1.0 | 103.9 ± 4.1 | 90.1 ± 4.0* | 101.6 ± 4.1 |
| | | 3.0 | 108.3 ± 3.1 | 89.0 ± 3.6* | 97.7 ± 2.5 |

(*$p < 0.05$, **$p < 0.005$, ***$p < 0.001$ compared to respective C.)

Plasma extravasation was measured by the Evans blue technique. CGRP (30 μg/kg, i.v.) showed only a 4-6 fold increase in plasma extravasation whereas equimolar doses of SP (10 μg/kg, i.v.) caused a 20-30 fold increase in plasma extravasation (nasal mucosa, conjunctiva, ureter, trachea). In conclusion, at equimolar doses CGRP is 10 times more potent than SP in producing vasodilatation whereas it causes only a modest plasma extravasation compared to SP.

259.16 CARDIOVASCULAR EFFECTS PRODUCED BY MICROINJECTIONS OF ATRIAL NATRIURETIC FACTOR (ANF) INTO THE AV3V REGION OF THE RAT BRAIN. M. A. Sills and D. M. Jacobowitz. Laboratory of Clinical Science, NIMH, Bethesda, MD 20205.

During the past few years, increased attention has focused on a new class of peptides referred to as atrial natriuretic factor (ANF), which was initially discovered in extracts from atrial tissue. Studies have shown that ANF possesses potent natriuretic and diuretic activity. In addition, ANF produces vasodilatation resulting in decreased blood pressure. Recent studies have reported that ANF-containing neurons exist in rat brain (Neuroendocrinology, 40: 92, 1985). Of particular interest was the finding that the greatest density of cells was located in the anteroventral third ventricle (AV3V) area of the brain. This region of the brain is currently thought to play a major role in blood pressure regulation, fluid and electrolyte balance. Based on these findings, we were interested in examining the effect of microinjections of ANF into the AV3V region of the brain to determine whether it may play a role in cardiovascular regulation.

In the present study, 50-100 nanoliters of saline or atriopeptin III (2-40 pmol) was injected into the rostral portion of the preoptic suprachiasmatic nucleus (posc) using a double-barreled glass micropipette. Rats were initially injected with saline, followed by ANF 30 minutes later. The results indicate that injections of 2 or 4 pmol of ANF elicit a modest rise in systolic and diastolic pressures, and a small increase in heart rate (HR). Specifically, 4 pmol ANF produced significantly greater effects than saline on systolic, diastolic and mean arterial blood pressures (BP). These values were increased over baseline values by 16 ± 2 (14%), 10 ± 2 (14%), and 12 ± 2 (14%) mm Hg, respectively, by ANF. HR was moderately increased by 26 ± 6 (7%) beats per minute (bpm) by this concentration of ANF. In contrast to these results, higher concentrations of ANF (20-40 pmol) produced substantial increases in both BP and HR. Injection of 20 pmol of ANF into the posc produced significantly greater effects than saline on systolic, pulse and mean arterial pressures, as well as on HR. These values were increased with respect to baseline values by 18 ± 3 mm Hg (15%), 12 ± 3 mm Hg (25%), 10 ± 1 mm Hg (12%), and 74 ± 10 (22%) bpm, respectively, by ANF. Injections of 50-100 nl of saline into the posc produced no significant change in either BP or HR. The onset of effects on BP and HR produced by ANF was seen 30-60 min after injection. Peak effects were usually observed approximately 90-120 min after onset, and the duration of the effect was 3-4 h, after which time values returned to baseline. In conclusion, these studies provide evidence for a functional role of ANF in the AV3V area of the brain, and lends support to the idea that it may play an important role in central cardiovascular regulatory mechanisms.

260.1 FURTHER PURIFICATION AND CHARACTERIZATION OF BOVINE DOPAMINE ($D_2$) RECEPTORS. Jai Ramwani* and Ram K. Mishra. Depts. of Psychiatry & Neurosciences, McMaster University, Hamilton, Ontario, L8N 3Z5. (Sponsored by Sandra Witelson.)

Using haloperidol-linked affinity sepharose gel, we reported our preliminary findings on the purification of dopamine ($D_2$) receptors (Fed. Proc. 41, 1325, 1982).

In this communication, we now report further purification to 2000 fold, and detailed characterization of the purified dopamine ($D_2$) receptors.

Bovine striatal crude mitochondrial-microsomal $P_2$-$P_3$ membrane preparation was solubilized by 0.25% cholic acid - 1M NaCl (final concentration) in .05 M Tris, 1 mM EDTA and 0.1 mM Dithiothretol buffer (pH 7.4) as described earlier (Prog. Neuro. Psycho. Pharmacol. and Biol. Psychiat. 7, 769, 1983).

Haloperidol was convalently linked to epoxy-activated sepharose CL-6B in dimethylformamide. Solubilized receptors were passed slowly at 4-6 ml/hr. over the Hal-linked sepharose. Bound receptors were eluted with 100 nM spiroperidol in 50 mM Tris 1 mM EDTA buffer containing .02% cholic acid. Bovine phospholipids were added to the eluting buffer to prevent receptor denaturation. Eluting agent was separated by concentrating the eluent through Amicon CF25 cones and passing over the G-25 column.

Affinity eluted receptors exhibited dopamine ($D_2$) receptor characteristics. Pharmacologically, purified receptors revealed similar affinity and specificity as membrane-bound $D_2$ receptors.

Scatchard analysis showed the $K_D$ value of 0.5 nM and $IC_{50}$ values for different agents were comparable to the membrane-bound receptor (Table below).

EFFECT OF VARIOUS AGENTS ON $^3$H-SPIROPERIDOL BINDING

| Agents | Membrane Receptor | Solubilized Receptor $IC_{50}$ nM | Affinity Purified $IC_{50}$ nM |
|---|---|---|---|
| Spiroperidol | 1.00 | 0.84 | 0.70 |
| D-Butaclamol | 4.00 | 3.99 | 9.66 |
| Haloperidol | 10.75 | 7.00 | 24.0 |

The $IC_{50}$ values (conc. of drugs that inhibits 50% of the $^3$H-spiroperidol binding) were obtained by using 7 different concentrations of drugs ($10^{-10}$ - $10^{-4}$ M) and were calculated by log probit analysis.

Proteins in the solubilized and purified receptor were estimated by the Bio-Rad method.

(Supported by MRC Canada and Ontario Mental Health Foundation.)

260.2 SUBCELLULAR LOCALIZATION OF THE SCH23390 RECEPTOR: DISSOCIATION FROM DOPAMINE-STIMULATED ADENYLATE CYCLASE. D.W.Schulz, E.J.Stanford*, and R.B.Mailman. Depts. Pharmacology and Psychiatry, Univ. North Carolina Sch. of Med., Chapel Hill, NC 27514.

The compound SCH23390 has generated great experimental interest because it is potent as both a $D_1$ dopamine receptor antagonist and an inhibitor of motor behaviors caused by dopamine agonists. Since such behaviors previously had been linked to activation of $D_2$ dopamine receptors, it is important to determine if the behavioral effects of SCH23390 are mediated by a direct action at $D_1$ sites, an indirect modulation of $D_2$ sites, or an interaction with a population of receptors which is not linked to adenylate cyclase activation. In order to investigate these possibilities, we have performed subcellular fractionation studies using rat caudate-putamen, and analyzed the distribution of the specific binding of [$^3$H]-SCH23390.

Initial experiments utilized the methods of Laduron et al. (J. Neurochem., 41:84, 1983). Freshly dissected striatal tissue was separated into 5 fractions: nuclear (N), heavy (M) and light (L) mitochondrial, microsomal (P), and supernatant (S). Markers used to assess the purity of M, L, P, and S were: cytochrome oxidase, [$^3$H]-dopamine uptake, 5'-nucleotidase, and dopamine presence, respectively. Assays for [$^3$H]-SCH23390 and [$^3$H]-spiperone binding and for dopamine stimulation of adenylate cyclase were performed for each fraction.

[$^3$H]-SCH23390 binding was present in all particulate fractions, and was greatest in the microsomal fraction. However, dopamine-stimulated adenylate cyclase activity was concentrated most heavily in the mitochondrial fractions. Interestingly, the distribution of [$^3$H]-SCH23390 binding closely paralleled that of [$^3$H]-spiperone. Additional experiments employing isopycnic centrifugation in continuous sucrose gradients were carried out in order to further resolve these distribution profiles.

These data provide preliminary evidence that there may be a component of [$^3$H]-SCH23390 binding which does not coincide with the dopamine receptor that is linked with adenylate cyclase activation. In addition, findings from these studies will be used to develop strategies for purifying the SCH23390 receptor.

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260.3 PHOTOAFFINITY LABELING AND PURIFICATION OF THE RAT STRIATAL D-2 DA RECEPTOR. J.Y. Lew*, E. Meller*, and M. Goldstein, Department of Psychiatry, New York University Medical Center, New York, NY, 10016.

The photoaffinity probe $^3$H-7 Azido-fluphenazine ($^3$H-AF) was used to label rat striatal DA receptors. Striatal membranes were incubated with 10 nM $^3$H-AF and the mixture was irradiated with a high pressure Hg lamp. The unreacted material was removed by washing the membranes with Tris-HCl buffer, pH 7.7, and the photolabeled membrane bound proteins were solubilized and purified. The $^3$H-AF photolabeled membrane bound proteins were solubilized with 10 mM CHAPSO and adsorbed on a wheat germ agglutinin (WGA) agarose column. The glycoproteins were eluted from the column with 0.2 M N-Acetyl glucosamin and subsequently electrophoresed on a 6%/SDS-polyacrylamide gel. Photolabeling with $^3$H-AF resulted in the covalent incorporation of the probe into several minor and one major peptide band with a molecular weight of approximately 92,000. Incubation of the striatal membranes with the irreversible DA receptor antagonist, EEDQ ($5 \times 10^{-4}$M) prevented the covalent labeling with $^3$H-AF of this peptide. The incubation of the membrane with $5 \times 10^{-4}$M (±) sulpride protected the DA receptors from inactivation by EEDQ ($5 \times 10^{-4}$M) and restored the covalent labeling of the 92,000 peptide with $^3$H-AF. These results indicate that the $^3$H-AF labeled peptide with the molecular weight of 92,000 represents a sub-unit of the D-2 DA receptors.

260.4 PROPERTIES OF $^3$H-N-METHYL SPIPERONE BINDING TO HUMAN AND RAT $D_2$ AND 5-$HT_2$ RECEPTORS IN VITRO

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N-methyl-spiperone (NMSP), containing $^{11}$C, has been successfully used as a Positron Emission Tomography ligand in humans (1). The signal detected in human caudate and putamen has been identified as primarily due to a $D_2$ receptor interaction and the signal detected due to a $D_2$ receptor interaction and the signal in the frontal cortex has been identified as primarily due to a 5-$HT_2$ receptor interaction. The study to be presented was designed and executed to obtain detailed information on the molecular interaction between N-methyl-spiperone and brain $D_2$ and 5-$HT_2$ receptors. As the half-life of $^{11}$C renders it difficult to use for detailed in vitro studies, a tritiated form of N-methyl-spiperone was synthesized (New England Nuclear).

Rat striatal, rat frontal cortex, human caudate, and human frontal cortex homogenates were prepared in a buffer of 50 mM Tris-HCl, pH 7.4 at 37°C. In the rat striatal and human caudate tissue saturation and kinetic studies were performed in the same buffer plus 140 mM NaCl; the NaCl was used because sulpiride ($10^{-5}$M) was used to define specific binding to $D_2$ receptors and sulpiride is a sodium dependent ligand (2). In rat and human frontal cortex tissue $10^{-5}$M cinanserin was used to define specific binding to 5-$HT_2$ receptors; evidence will be presented confirming the 5-$HT_2$ receptor specificity of this signal in both the rat and human tissues. Table one displays the preliminary equilibrium dissociation constants and kinetic rate constants for $^3$H-N-methyl-spiperone binding to $D_2$ and 5-$HT_2$ receptors.

| | $B_{max}$ | $K_d$ | $k_{on}$ | $k_{off}$ |
|---|---|---|---|---|
| | | $D_2$ | | |
| Rat | 14.4 pmol/g | 0.09nM | $1.71 \times 10^9$ | $1.20 \times 10^{-1}$ |
| Human | 5.0 pmol/g | 0.05nM | | |
| | | 5-$HT_2$ | | |
| Rat | 12.4 pmol/g | 0.24nM | $1.26 \times 10^9$ | $1.47 \times 10^{-1}$ |
| Human | 5.7 pmol/g | 0.26nM | | |

Data from completed saturation and kinetic studies as well as detailed pharmacological analysis of the $^3$H-NMSP signal in rat and human tissues will be presented.

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260.5 AGE AND DOPAMINE $D_1$ AND $D_2$ RECEPTORS IN HUMAN BRAIN.

Natalie H. BZOWEJ* and Philip SEEMAN. Dept. of Pharmacology, University of Toronto, Toronto, Canada M5S 1A8.

Older people exhibit spontaneous oral dyskinesias which may stem from changes in their brain dopamine receptor densities. Different findings, however, have been reported for the relation between age and the density of $D_2$ dopamine receptors in human brain. Severson et al. (1982) found a fall of 2.5% per decade in two series of human caudate nuclei (N=13;N=13), but no such fall in a third series of caudates (N=17) or in 3 series of human putamens. Wong et al. (1984) found less binding of a single dose of $^{11}$C-N-methylspiperone in males over 40 yrs. Seeman et al. (1984) found little relation between age and $D_2$ density. Thus, we extended our series for $D_2$ densities and also measured $D_1$ densities.

The $D_1$ receptor density ($B_{max}$) was measured by receptor-saturation-type experiments using a range of 100 to 4000 pM $^3$H-Schering-23390 (Seeman et al., 1985). The $D_2$ density was measured using 10 to 1000 pM $^3$H-spiperone. The $B_{max}$ values were determined by Scatchard analysis. Non-specific binding for $D_1$ and $D_2$ was defined as that binding inhibited by 1 uM (+)-butaclamol.

The $B_{max}$ values (pmoles/gm wet tissue) were:

| AGE, | $D_2$ | | $D_1$ |
|---|---|---|---|
| years | Caudate | Putamen | Striatum |
| <40 | 12.2 N=10 | 12.3 N=9 | 14.5 N=9 |
| 40-69 | 10.5 N=35 | 10.9 N=35 | 12.2 N=14 |
| ≥70 | 10.0 N=36 | 13.0 N=36 | 12.1 N=11 |

We found that the $D_2$ dopamine receptor densities were constant with age in both caudate and putamen. No difference existed between males and females. $D_1$ receptors in striatum were also constant with age. (Supported by the Ont. Ment. Health Fdn.)

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260.6 **QUANTITATIVE AUTORADIOGRAPHY REVEALS THAT DOPAMINE (D-2) RECEPTORS ARE LOCATED ON NEURONS INTRINSIC TO THE CAUDATE-PUTAMEN.**

**J.N. Joyce and J.F. Marshall. Dept. of Psychobiology, Univ. California Irvine, Irvine, CA 92717.**

**As part of our investigations of the lateral to medial gradient of D-2 receptors in the rat caudate-putamen (CPU) (Joyce, J.N. et al. Brain Res., 1985), we used quantitative autoradiography to determine the density of [$^3$H]spiroperidol sites in rat CPU after unilateral injection (90 nmol/0.5 µl, 15 min) of quinolinic acid (QA) into this structure or after ablation of neocortical regions. Rats were sacrificed at 7 or 21 days postoperatively, and brains sectioned and processed for [$^3$H]spiroperidol (0.1 - 1.0 nM, 100 Ci/mmol, Amersham) binding to thin sections in the presence of 40 nM ketanserin with or without 1 µM (+)-butaclamol. The tissue and a set of $^3$H-standards were apposed to $^3$H-sensitive film for 1-3 weeks. Autoradiographs were digitized, linearized and the density of specific [$^3$H]spiroperidol binding read from regions of CPU with the aid of an image processor (Altar, C.A. et al. Neurosci. Meth., 100:173, 1984). Quantification of the zone of QA-induced cell loss was achieved by acetylcholinesterase (AChE) histochemistry (following DFP treatment) and thionin staining of adjacent sections. At the site of total loss of large AChE-positive neurons D-2 sites were decreased by as much as 94% by 21 days post-op. The affinity of [$^3$H]spiroperidol for the sites was unchanged in the QA-injected CPU. In other animals, given ablations of specific neocortical fields (medial prefrontal, motor, somatosensory) or of the entire parietal-frontal cortex of one hemisphere, sections were processed for quantitative autoradiography, scraped off for scintillation counting, or thionin-stained. Analysis of the 21 day post-op tissue showed that unless there was accompanying damage to the CPU, there was no loss of D-2 sites in the CPU. The two sets of results lead to the hypothesis that D-2 sites are primarily located on neurons intrinsic to the CPU, and are not localized to neocortical afferents. Values for the regional density of D-2 sites correlate very highly with regional CAT activity and high-affinity choline uptake (Joyce, J.N. and J.F. Marshall Neurosci. Lett., 53:127, 1985), suggesting a preferential relationship of this dopamine receptor with striatal cholinergic neurons.**

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260.7 KINETIC MODELING OF THE BINDING OF NEUROLEPTIC DRUGS TO BRAIN RECEPTORS DETERMINED BY POSITRON EMISSION TOMOGRAPHY.

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From positron emission tomography (PET) studies with appropriately labeled neuroleptics, the distribution of radionuclides in various brain structures can be followed over a period of several hours. This allows an in vivo determination of the kinetics of the binding of these drugs to neuroleptic receptors. The application of specific models to these data provides information about the kinetic constants associated with the binding of drug to receptor. We have modeled data from PET studies using $^{18}$F-labeled spiroperidol, benperidol and N-methylspiroperidol in the baboon and N-methylspiroperidol in humans.

The parameters governing the uptake and loss of drug were determined from regions with few or no receptors, such as the cerebellum, and from baboon experiments in which specific receptor binding was blocked by pretreatment with the potent neuroleptic (+)-butaclamol.

Frequent arterial blood sampling, along with measurement of the fraction of plasma radioactivity due to unmetabolized radioligand, allowed an accurate assessment of the input function for brain radioactivity in these studies. Without this correction for metabolism, the model overestimated cerebellar activities for times greater than 30 min after injection. The simplest model used for receptor-containing regions was a single-site model. While this model was capable of describing the major part of the activity over the course of the experiment, in several cases it underestimated activity for times less than 2 h after injection. This suggests that the binding process may be more complex than can be represented by a single-site receptor model. From computer simulation studies we have shown that receptor concentrations can be determined by two experiments, one at a high specific activity radioligand, which defines the upper limit of the ligand distribution curve, and a second experiment at a lower specific activity radioligand such that the ligand distribution curve is between that of the high specific activity case and that characteristic of brain regions with few or no receptors. PET baboon studies using radioligand at low specific activity have demonstrated the feasibility of this approach to determining receptor concentrations in vivo.

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260.8 INVESTIGATION OF INHIBITORY POTENCIES OF VARIOUS PSYCHOTROPIC DRUGS ON D-1 AND D-2 DOPAMINE RECEPTORS

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We investigated inhibitory effects of various psychotropic drugs on two different dopamine receptors, D-1 and D-2, using receptor binding assay and (-)-apomorphine induced stereotypy. Clinical potencies and laboratory behavioral effects have so far been evaluated mainly in terms of D-2 receptor, but very little of D-1 receptor. The present studies were therefore focussed on D-1 receptor.

In binding study, we determined $IC_{50}$ values of drugs on D-1 and D-2, using $^3$H-cis-flupenthixol and $^3$H-spiperone bindings in rat striatum. SCH23390, cis-flupenthixol and fluphenazine showed high affinities for D-1 receptor ( $IC_{50}$ 0.9nM, 0.6nM, 4nM respectively ), other phenothiazine derivatives and amoxapine showed moderate affinities ( perphenazine,30nM, chlorpromazine,50nM, thioridazine,39 nM, amoxapine,63nM ). But butyrophenone derivatives and YM-09151-2 indicated markedly low affinities for D-1 receptor ( spiperone,560 nM, moperone,300nM, haloperidol,140nM, YM-09151-2,1800nM ) in contrast with high affinities for D-2 receptor ( spiperone,0.16nM, moperone,6.3nM, haloperidol,3.2nM, YM-09151-2,0.14nM ). SCH23390 was kindly gifted from Dr. A. Bennet.

Recently, Goldman and Kebabian ( Mol.Pharmacol.,1983 ) reported that (-)-apomorphine stimulates both D-1 and D-2 receptors, whereas (+)-apomorphine blocks those receptors. They also showed that the affinity of (-)-apomorphine toward D-2 receptor was much greater than that toward D-1 receptor. Until now most behavioral tests with apomorphine in rat have been done using relatively small dose of (±)-apomorphine. We therefore investigated whether or not the stereotypies induced by high dose of (-)-apomorphine in rat related with D-1 receptor.

Continuous stereotypic biting or licking was induced constantly in rat by 10mg/kg (-)-apomorphine(i.p.). Fluphenazine, perphenazine and haloperidol had widely different inhibitory effects as shown in the table, although these drugs had similar affinities for D-2 and these penetration rates into CNS were thought to be not largely different from the data of cataleptogenetic effects. The differences were more marked when scopolamine(2mg/kg i.p.) was given concomitantly. And D-1 selective antagonist, SCH23390, showed potent inhibitory effect. These results suggest the possibility that the stereotypies induced by high dose of (-)-apomorphine are mediated through dopamine receptors containing D-1.

| | APO-stereotypy without scopo. $ED_{50}$(95% CL) umol/kg i.p. | APO-stereotypy with scopo. $ED_{50}$(95% CL) umol/kg i.p. |
|---|---|---|
| fluphenazine | 0.28 ( 0.18 - 0.43 ) | 1.35 ( 0.92 - 1.98 ) |
| perphenazine | 3.8 ( 2.7 - 5.3 ) | 13.0 ( 10.4 - 16.3 ) |
| haloperidol | 3.9 ( 3.4 - 4.3 ) | 28.0 ( 20.7 - 37.8 ) |
| SCH23390 | 1.2 ( 0.95 - 1.6 ) | 2.0 ( 1.48 - 2.7 ) |

260.9 DIFFERENCES IN MIDBRAIN DOPAMINE CELL AUTORECEPTOR SENSITIVITY IN GENETIC MOUSE STRAINS. B.S. Walker, D.C. German and M.K. Sanghera, Depts. of Psychiat. and Physiol., U. of Texas Health Sci. Cntr., Dallas, TX. 75235.

Genetically inbred strains of mice have been found to possess differing numbers of (a) midbrain dopamine (DA) neurons (Fink & Reis, Brain Res., 222:335-345, 1981) and (b) postsynaptic DA-D2 receptors in striatal and limbic regions (Helmeste & Seeman, Psychiat. Res., 7:351-359, 1982). In our laboratory, we have been examining the anatomy, electrophysiology and pharmacology of midbrain DA neurons in the BALB/c and CBA mouse strains. We have reported differences in midbrain DA cell densities, DA turnover rates in the caudate and nucleus accumbens (German et al., Neurosci. Abstr., 9:1150, 1983), and dopamine neuronal firing rates (Sanghera & German, Neurosci. Abstr., 10:950, 1984) in these mouse strains.

The purpose of the present study was to compare the sensitivity of DA cell body autoreceptors in mice which contain different numbers of midbrain DA neurons. Extracellular single cell recordings were made from DA neurons in the substantia nigra zona compacta (nucleus A9) and the ventral tegmental area (nucleus A10) in chloral hydrate anesthetized BALB/c and CBA mice. Recording sites were marked by the iontophoretic ejection of Fast Green dye from the glass recording micropipettes. A total of 20 single DA neurons were recorded from A9 and A10 areas in the two strains. As previously reported, DA cells in the CBA fired significantly faster than in the BALB/c, (4.1 ± 0.4 vs. 2.6 ± 0.5 impulses/sec). Intravenous administration of the DA agonist apomorphine (APO; 0.5-4.0 µg/kg) inhibited DA neuronal activity in both strains and this inhibition could be reversed with low doses of the DA antagonist, haloperidol (0.1 mg/kg). However, DA neurons in the BALB/c mice were significantly more sensitive to low doses of APO (0.5-1.0 µg/kg) than the CBA mice, irrespective of differences in baseline firing rates. At higher doses, DA cells in both strains were equally sensitive to the inhibitory effects of APO.

In conclusion, we find that: (1) firing rates of DA neurons in the A9/A10 regions are higher in the CBA strain than in the BALB/c; (2) DA cell body autoreceptors in mice are considerably more sensitive to APO when compared to rat autoreceptors ($ID_{50}$ for mice = 3.0 µg/kg vs. 9.3 µg/kg for rat); and (3) in the CBA mice, which have fewer DA neurons and higher baseline firing rates, DA neurons have less sensitive cell body autoreceptors than in the BALB/c mice. Research supported by NIMH grants MH-30546 and MH-39699.

260.10 CHARACTERIZATION OF D2 DOPAMINE RECEPTORS IN THE MEDIAL FRONTAL CORTEX OF THE RAT. A.J. MACLENNAN*, A. JAKUBOVIC AND H.C. FIBIGER. Div. of Neurol. Sci., Dept. of Psychiat., U.B.C., Vancouver, B.C. Canada V6T 1W5.

The demonstration of a dopaminergic projection innervating the frontal cortex (FC) has led to many anatomical, electrophysiological, biochemical and behavioral studies of this system. Much of the biochemical and behavioral work has indicated that the FC dopamine (DA) system is functionally distinct from subcortical DA systems. Additionally, research has implicated the FC DA system in the pathology of some forms of schizophrenia. However, characterization of D2 DA receptors in the FC has been impeded by methodological difficulties associated with the small number of potential D2 binding sites in this area. In the present study these problems were attenuated by employing a low concentration of [$^3$H]-spiperone (25pM), by occluding serotonin (5-HT) receptors with 10nM ketanserin in each incubation, by using only the DA innervated area of the medial frontal cortex (MFC), and by reducing the variability associated with nonspecific and filter binding. In competition studies the specific binding (defined by 10µM (-)-sulpiride) displayed the characteristics of D2 receptors. Phentolamine, naloxone, baclofen, atropine and clonazepam were ineffective in the nM range. (-)-sulpiride was more potent than (+)-sulpiride. DA was more potent than 5-HT or norepinephrine. The specific binding was inhibited with nM concentrations of neuroleptics. The potencies of the neuroleptics correlated with their in-vivo potencies as antipsychotics as well as antagonists of DA-related behaviors. The potencies of all drugs tested with MFC tissue correlated with their potencies in an identical assay using the striatum (Str). Apomorphine was exceptional in that it inhibited MFC binding more than Str binding. This suggests that there may be proportionally more "D2 high" sites in the MFC compared to the Str.

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260.11 CHARACTERIZATION OF DOPAMINE RECEPTORS ON CULTURED NEURONS FROM VENTRAL MESENCEPHALON OF MOUSE E.J. Heyer, E. Chung, M. Van Woert, and J. Parise*. Department of Neurology, Mount Sinai School of Medicine, New York, New York 10029.

Primary dissociated cell (PDC) cultures of ventral mesencephalon (VM) from mouse were prepared as a model to study dopamine actions. In vivo, dopaminergic neurons are located in substantia nigra zona compacta, as well as in ventral tegmental area. In substantia nigra zona compacta dopamine is released from dopaminergic neuron dendrites. In this study we have characterized the dopamine receptor subtype found on cells in these cultures by determining whether there is dopamine stimulated adenylate cyclase activity present (mediated by the D-1 receptor) and whether the high affinity D-2 antagonist spiroperidol binds to cultured cells (mediated by the D-2 receptor if no dopamine stimulated adenylate cyclase is present).

PDC cultures of VM and PDC cultures of spinal cord were prepared from 12-14 day old fetal mice. Dopamine stimulated adenylate cyclase activity was determined by measuring the formation of $^{32}$P-cAMP from $^{32}$P-ATP in cells grown in PDC cultures of VM and in adult female mouse striatum. $^3$H-spiroperidol (1 nM) receptor binding and $^3$H-flupenthioxol (1 nM) receptor binding were performed on homogenized cells removed from 35 mm culture dishes of VM or spinal cord cultures by incubating with 1mM EDTA for 5 minutes; subsequently calcium chloride (5mM) was added back. Butaclamol (10uM) or 5-hydroxytryptamine (5-HT) (10uM) was used to determine nonspecific binding.

Dopamine (5uM) failed to stimulate adenylate cyclase activity in cells from PDC cultures of VM, whereas this concentration of dopamine produced stimulation of adenylate cyclase by 55% in adult mouse striatal tissue. Therefore, the D-1 receptor subtype associated with adenylate cyclase is not present in cells from VM. Binding was specific for a dopamine receptor site and was not contaminated by a serotonin receptor site since butaclamol but not 5-HT displaced $^3$H-spiroperidol binding. The D-2 receptor antagonist spiroperidol bound with high affinity to cells from PDC cultures of VM but not to cells of PDC cultures of spinal cord, a region in the intact animal where little to no dopamine receptor binding is expected. Therefore, cells from cultured VM have D-2 receptors. D-3 receptors which also fail to activate adenylate cyclase but bind flupenthixol with high affinity and spiroperidol with low affinity are not present in cultured VM since there was no flupenthixol binding in these cultures. Spiroperidol binding increased with age reaching a maximum at three to four weeks in culture and remained constant for at least twelve weeks. Scatchard and Hill analysis performed on cells grown in culture for at least three weeks showed $K_D$ of 1.22 nM, $B_{max}$ of 66.8 fmole/mg of protein and Hill coefficient of 1.0.

In summary, PDC cultures of VM are a useful model to study the action of the D-2 dopamine receptor subtype.

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260.12 INHIBITION OF $K^+$-STIMULATED [$^3$H]DOPAMINE (DA) AND [$^{14}$C]ACETYLCHOLINE (ACH) RELEASE BY THE PUTATIVE DA AUTORECEPTOR AGONIST, B-HT 920. C.J. Schmidt*, A. Lobur, and W. Lovenberg. (SPON: F. Porreca). Section on Biochem. Pharmacol., NHLBI, NIH, Bethesda, MD 20205.

Results from a number of recent studies suggest that the azepine derivative, B-HT 920 (2-amino-6-allyl-5,6,7,8-tetrahydro-4 H-thiazolo-[4,5-d]-azepine dihydrochloride), behaves as a dopamine (DA) agonist with a high selectivity for the DA autoreceptor in vivo. Behavioral (Anden et al., N-S Arch. Pharmacol. 321:100, 1982) and neurochemical (Anden et al., J. Neurol. Trans. 58:143, 1983) studies have shown B-HT 920 to be a potent inhibitor of DA turnover by a mechanism which is sensitive to haloperidol. However, even high doses of the drug do not elicit increased locomotor activity or stereotypy (Brown and Mitchell, Proc. Brit. Pharmacol. Soc. Abst. p. 155, 1984). The presynaptic selectivity of B-HT 920 was examined by dual label experiments using rat neostriatal slices incubated with [$^3$H]DA and [$^{14}$C-methyl]choline to preload DA and ACh terminals respectively. Slices mounted on nichrome screens were serially transferred through a sequence of LS vials containing Kreb's-Ringer bicarbonate buffer (KRB). Release during the 5-min incubation in each vial was determined by liquid scintillation counting. The effect of B-HT 920 on the release of labeled DA and ACh as evoked by 20 mM $K^+$/KRB was taken as an indication of the activation of presynaptic and postsynaptic DA receptors, respectively. B-HT 920 inhibited the $K^+$-stimulated release of both transmitters in a concentration-dependent manner in the µmolar range. This effect of B-HT 920 was totally blocked by the active (+) isomer of the DA antagonist, butaclamol, while the (-) isomer was without effect. The selective $D_2$ blocker, sulpiride, also antagonisted B-HT 920's inhibition of [$^3$H]DA and [$^{14}$C]ACh release. In contrast, tolazoline, an alpha adrenoceptor blocking agent, did not block the effect of B-HT 920. B-HT 933, a structural analogue of B-HT 920 reported to lack DA autoreceptor affinity in in vivo models, was without effect on the $K^+$-stimulated release of either [$^3$H]DA or [$^{14}$C]ACh. B-HT 920 was without effect on the $K^+$-stimulated release of [$^3$H]5HT from the same slices from which it inhibited the release of [$^{14}$C]ACh. The results show that B-HT 920 selectively inhibits the release of both [$^3$H]DA and [$^{14}$C]ACh in vitro, specifically by activation of DA receptors, probably of the $D_2$ subtype. Since these results indicate an activation of postsynaptic DA receptors by B-HT 920 in vitro, further studies may be required to elucidate its mechanism of action in the intact animal.

260.13 RESTORATION OF DOPAMINERGIC INHIBITION OF CHOLINERGIC FUNCTION AFTER PARTIAL DAMAGE TO THE NIGROSTRIATAL BUNDLE. R.G. MacKenzie, M.K. Stachowiak, E.M. Stricker, and M.J. Zigmond. Dept. of Biological Sciences, Center for Neurosciences, Univ. of Pittsburgh, Pittsburgh, PA 15260.

While acute, pharmacological disruption of transmission at dopamine (DA) synapses in brain causes akinesia, after permanent lesions of DA systems only the near-total loss of DA elicits such behavioral dysfunctions. Moreover, when these deficits occur they usually are transient. To examine this phenomenon, we have studied the effects of destroying DA neurons on the capacity of endogenous DA to inhibit acetylcholine (ACh) release in striatum of adult male rats.

Striatal slices were preincubated in Krebs bicarbonate buffer with 50 nM [$^3$H]-choline, washed with buffer containing $10^{-5}$M hemicholinium, and then superfused with that buffer for 60 min at 0.2 ml/min, after which collection of 5-min fractions was begun. Slices were stimulated via platinum mesh electrodes with biphasic square-wave pulses (8 Hz, 2.5 msec duration, 20 mA) for 1 min beginning at 25 min ($S_1$) and 75 min ($S_2$) and $CA^{2+}$-dependent, fractional release of [$^3$H]-ACh was measured. Under control conditions, [$^3$H]-ACh release at $S_1$ and $S_2$ were the same ($S_1/S_2$, 0.96 $\pm$ 0.06, n=16). Addition of $10^{-6}$ M of the DA antagonist (S)-sulpiride beginning at 40 min increased $S_2/S_1$ to 2.02 $\pm$ 0.09 (n=27), consistent with previous suggestions that endogenous DA exerts an inhibitory influence on ACh release. When slices were taken from rats treated 3 days earlier with 6-hydroxydopamine (250 ug, ivt.) the effects of sulpiride were decreased ($S_2/S_1$, 1.41 $\pm$ 0.13, n=4) if the depletions were >80%. In contrast, when lesions were less severe (50-70% depletion of DA) the effects of sulpiride were the same as in control slices ($S_2/S_1$, 2.05 $\pm$ 0.15, n=4). Moreover, when animals with the more extensive lesions were given 2 months to recover from surgery, even those with DA depletions of 90% exhibited normal inhibition of ACh by endogenous DA ($S_2/S_1$, 2.27 $\pm$ 0.19, n=6). In a separate experiment, the density of striatal DA receptors was measured using [$^3$H]-spiperone 2 months post-lesion. An increase in D2 sites was observed only in animals exhibiting a DA depletion of 80% or greater. Collectively, these observations parallel our previous behavioral findings and suggest that there is a large capacity for increased DA function in striatum. Some of this reserve capacity may arise from the immediate decrease of DA reuptake after degeneration of DA terminals and the enhanced release of DA from the spared terminals. When those reserves are exhausted, additional adaptive responses such as receptor up-regulation may be recruited. (Supported by NS 19608.)

260.14 17β-ESTRADIOL AT A PHYSIOLOGICAL DOSE ACUTELY INCREASES RAT BRAIN DOPAMINE TURNOVER. T. Di Paolo[1], C. Rouillard*[2] and P. Bédard[2], [1]School of Pharmacy, Laval Univ., Quebec G1K 7P4 and Dept. of Molecular Endocrinology, Laval Univ. Hosp. Center, Quebec G1V 4G2; [2]Neurobiology Lab., Dept. of Anatomy, Laval Univ., Quebec G1J 1Z4, Canada.

We have investigated the effect of an acute physiological dose of 17β-estradiol on dopamine (DA) metabolism in the rat striatum and nucleus accumbens. Plasma 17β-estradiol and prolactin concentrations were measured by specific radioimmunoassays while brain dopamine and its metabolites were assayed by HPLC with electrochemical detection. One injection of 17β-estradiol (30 ng s.c.) to ovariectomized rats leads to a significant elevation of this steroid concentration in the plasma after 15 min with a peak at 30 min and a return to control values at 45 min while plasma prolactin concentrations are not significantly modified. This concentration of 17β-estradiol leads to a rapid surge of about 100 pg/ml of plasma estradiol concentrations and is similar to the proestrus blood estradiol peak which is in the order of 50 pg/ml. Striatal dopamine concentrations remained essentially unchanged following the estradiol injection while the dopamine metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) and dopamine turnover as estimated with the ratios DOPAC/DA and HVA/DA are elevated 30 min after the steroid injection. A similar effect of the estrogen injection is observed in the nucleus accumbens where dopamine concentrations are unchanged while dopamine turnover is increased 30 min after the steroid injection. Increased dopamine turnover in the striatum and nucleus accumbens occurs in coincidence with peak plasma 17β-estradiol concentrations. Striatal dopamine receptor affinity ($K_D$) and density (Bmax) assayed with [$^3$H]spiperone binding remain unchanged following the injection of 30 ng of estradiol. In ovariectomized rats with a unilateral lesion of the entopeduncular nucleus, the same dose of 17β-estradiol induces a postural deviation to the lesioned side with a maximum at 30 min. These results suggest that very small doses of estradiol are able to increase dopamine turnover. This effect is seen within minutes and is relatively short. It is probably non-genomic, presynaptic and is similar to the effect of a small dose of a dopamine releasing agent. Supported by the Medical Research Council of Canada.

260.15 TRITIATED AGONIST BINDING TO A HIGH AFFINITY DOPAMINE RECEPTOR IN THE BOVINE RETINA. J.M. Ackerman* and M.E. Gnegy. University of Michigan, Ann Arbor, MI 48109.

Our previous studies suggested that light could regulate dopamine (DA)-sensitive adenylate cyclase activity in the retina (J. Neurochem., 42:1632, 1984). To further investigate the regulation of DA receptor activity in retina, we are developing an assay to measure DA receptor binding in the bovine retina using [$^3$H]DA and standard filter binding techniques. Our studies demonstrate that saturable, specific binding of [$^3$H]DA exists in retinal membrane preparations. Maximum binding occurs within forty-five minutes at 22°C, and the level of specific binding is 75%. Scatchard analysis of [$^3$H]DA demonstrates the presence of a high affinity binding site having a $K_d$ of 8.3nM and a $B_{max}$ of 690fmoles/mg protein. In addition, a low affinity component of binding is present with unestimable parameters due to high nonspecific binding.

We have measured competition by various DA agonists and antagonists for [$^3$H]DA binding sites, such as DA, ADTN, cis-flupenthixol, fluphenazine and butaclamol. The relative potencies for DA antagonists in competing for binding correlates with their ability to inhibit DA-stimulated adenylate cyclase. We have demonstrated stereospecificity of binding by competition experiments with the (+) and (-) isomers of butaclamol. The (+) isomer, which is a potent inhibitor of DA-stimulated adenylate cyclase, inhibits [$^3$H] DA binding with an $IC_{50}$ of 225nM while the (-) isomer, which is inactive, did not effectively inhibit [$^3$H]DA binding. Additional studies demonstrate that the dose-response curve for [$^3$H]DA binding in the retina displays a shift to the right by GppNHp, a non-hydrolyzable analog of GTP. This suggests the presence of a receptor linked to adenylate cyclase. The $IC_{50}$ for DA increases from 4.6 to 9.1nM and the total binding of [$^3$H]DA decreases more than two-fold in the presence of 50uM GppNHp.

In conclusion, we have demonstrated agonist binding to DA receptors in the retina with a high level of specific binding. Competition experiments with dopaminergic drugs indicate that binding is selective for DA receptors and that the binding is stereospecific. The evidence suggests that [$^3$H]DA binds to a high affinity D-1 dopamine receptor. In addition, it appears that binding occurs to a physiologically relevant receptor as evidenced by a decrease in receptor affinity in the presence of guanyl nucleotide.

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260.16 DOPAMINE-D2 RECEPTORS LOCATED ON PHOTORECEPTOR MEMBRANES COUPLE TO A PERTUSSIS- AND CHOLERA-TOXIN-SENSITIVE GTP-BINDING PROTEIN. M. R. Brann* and C. Jelsema*(SPON: P. St. John) Lab of Cell Biology, NIMH, Bethesda, MD 20205.

Neuroleptic agents exert their therapeutic efficacy by binding to dopamine(DA)-D2 receptors(R). For all forebrain structures, the density of DAergic innervation correlates with the density of D2 Rs. In the retina, light induces the synthesis and release of DA in neurons which are located in anatomical register with photoreceptors(PR). In PRs, light activates the enzyme cGMP-dependent-phosphodiesterase(PDE) by bleaching rhodopsin, which subsequently couples to a GTP-binding protein(transducin) that is sensitive to both pertussis(Pt)- and cholera(Ct)-toxin.

We have found that [$^3$H]spiperone binds to saturable sites on PR membranes isolated from bovine retina, exhibiting a $K_D$ 0.2 nM and a $B_{max}$ 1 pmole/mg protein when nonspecific binding is defined with (-)sulpiride(SUL). These sites are displaced by nM concentrations of apomorphine(APO), SUL and (+)butaclamol, but not by (-)butaclamol, prazocin and mainserin. These sites are identical to D2 Rs observed in other tissues under similar assay conditions. The density of D2 Rs correlates with the concentration of rhodopsin in several preparations of retinal membranes.

Ct- and Pt-toxin catalyze the ADP ribosylation of a 40 kDa protein in crude retinal- and PR-membranes. Both toxins also decrease the affinity of APO for the D2 R by the same magnitude as GTP and GppNHp. GTP and GppNHp have no effect on the affinity of APO for D2 Rs after toxin-treatment. Since transducin is abundant in PR membranes, and is sensitive to both Ct- and Pt-toxin, these data suggest that D2 Rs couple to the same GTP-binding protein as rhodopsin. This conclusion is supported by our observation that APO activates PDE in a manner which is blocked by SUL and (+)butaclamol (see abs. C. L. Jelsema, Y. Ishihara and M. R. Brann).

260.17 D-1 DOPAMINE (DA) RECEPTOR-MEDIATED RELEASE OF $^{3}$H-ACETYLCHOLINE (ACH) FROM RABBIT RETINA IN VITRO. J.G. Hensler and M.L. Dubocovich. Dept. Pharmacol., Northwestern Univ. Med. School, Chicago, IL 60611.

Two pharmacologically distinct dopamine (DA) receptor subtypes are found in the vertebrate retina: The D-1 DA receptor, linked to the activation of adenylate cyclase, and the D-2 DA autoreceptor, which inhibits the calcium-dependent release of DA via a negative feedback mechanism mediated by the neurotransmitter itself. The D-2 DA receptor is activated by nanomolar concentrations of DA and DA agonists such as pergolide, while activation of adenylate cyclase through D-1 DA receptors requires micromolar concentrations (Kebabian & Calne, Nature, 277, 93, 1979; Dubocovich & Weiner, J. Pharmacol. Exp. Ther., 219, 701, 1981; J. Pharmacol. Exp. Ther., in press). We report here the effect of DA and DA agonists on the release of ACh from rabbit retina. Retina pieces were labeled in vitro with $^{3}$H-choline (S. A.: 80 Ci/mmol) and superfused with Krebs' solution (1.3 mM calcium) containing hemicholinium-3 (10 uM). Samples of the superfusate were collected in 4-min fractions after the spontaneous outflow of tritium had leveled off. The release of tritium, expressed as percent of total tissue radioactivity released above spontaneous levels, was evoked by exposure to the DA agonists for one 8-min period (S). In the presence of the D-2 DA receptor antagonist S-sulpiride (1 uM), DA (1 uM -1 mM) evoked the release of tritium in a concentration-dependent manner. All other DA agonists were tested in the absence of S-sulpiride. The concentration of agonist necessary to induce 0.5% release of tritium was for pergolide, 0.2 uM; for DA, 14 uM; for R-SKF 38393, 16 uM; for LY 181990, 71 uM. Omission of calcium from the superfusion medium prevented the agonist-evoked release of tritium. The S-isomer of the selective D-1 DA receptor agonist SKF 38393 was significantly less potent to induce tritium release than the R-isomer. LY 181990, the stereoisomer of the selective D-2 DA receptor agonist LY 171555, did not inhibit the release of DA through D-2 autoreceptor activation, but evoked ACh release in a concentration dependent manner. The DA receptor antagonist alpha-flupenthixol (1 uM) antagonized the calcium-dependent release of ACh induced by pergolide. Beta-flupenthixol was significantly less potent. The selective D-1 DA receptor antagonist SCH 23390 (1 uM) antagonized the release of tritium evoked by DA. Release of ACh was also evoked by forskolin (10 uM, 30 min) suggesting the involvement of adenylate cyclase activation in this release process. These results suggest that in the rabbit retina activation of a stereoselective receptor with pharmacological characteristics of D-1 DA receptors induces calcium-dependent release of ACh, possibly through a cAMP-mediated process. USPHS grants EY 04788 and MH 09215.

260.18 EVIDENCE FOR A ROLE OF D-1 RECEPTOR STIMULATION IN THE GENERATION OF EEG ACTIVITY AND AROUSAL. Ongini,M.G. Caporali* and M. Massotti. Dept. of Pharmacology, Istituto Superiore di Sanità, Viale Regina Elena, 299 - 00161 Rome, Italy.

In the attempt to clarify the functional role of D-1 receptors, we studied the effects of the D-1 receptor agonist SKF 38393 on the EEG activity in the rabbit. Selectivity of action was assessed by studying the interaction with the D-1 antagonist SCH 23390 and the D-2 antagonist (-)-sulpiride. Investigation was then extended to the effects of apomorphine, a potent D-2 agonist with weak activity at D-1 receptors.

Adult male rabbits were implanted with electrodes for recording of EEG activity. SKF 38393 was injected at 1.25-10 mg/kg iv. The time spent in EEG desynchronization, as evidenced by low-amplitude fast frequency waves, was evaluated for 60 min after injection. SKF 38393 dose-dependently produced EEG desynchronization associated with arousal. No signs of stereotyped behaviors were observed. These effects were prevented by SCH 23390 (0.003-0.01 mg/kg iv), but not by (-)-sulpiride (25 mg/kg iv). Similar results were also obtained in the rat thus confirming that SKF 38393 specifically induces a dose-dependent increase of arousal Apomorphine, at the dose of 1 mg/kg iv, also induced EEG desynchronization. As observed for SKF 38393, the effects on the EEG were prevented by the same doses of SCH 23390, but not by (-)-sulpiride.

These results provide evidence that stimulation of D-1 receptors by SKF 38393 induces EEG desynchronization and arousal. Thus, it seems likely a functional role of D-1 receptors in the generation of EEG activity related with the state of arousal. The data indicate that also the effects on the EEG induced by high doses of apomorphine may be attributed to D-1 receptor stimulation.

## DEVELOPMENT AND PLASTICITY II: AGING

261.1 IMPAIRED SURVIVAL AND GROWTH OF FETAL SEPTAL NEURONS IN THE AGED CNS. J.P. Vicedomini, G.H. Jackson*, S.T. DeKosky and S.W. Scheff. Depts. of Anatomy and Neurology, Lexington, VA and Univ. of Kentucky Medical Center and Sanders-Brown Research Center on Aging, Lexington, KY 40536.

Lesion-induced neuronal plasticity diminishes with increasing age. The reduction may reflect changes in the growth capacity of the neurons and/or changes in the ability of the aged CNS to provide the appropriate supportive milieu. Tissue transplantion techniques may help to specify the nature of diminished reactive growth in the aged CNS. Fetal tissue transplanted into neonates or young adults survives and integrates with the host CNS. If the aged CNS provides the appropriate milieu for reactive growth then transplanted fetal tissue should integrate with the CNS of aged subjects as well as it does with young adults. If the milieu is not supportive the two age groups should show very different survival and integrative capacities.

Unilateral transection of the fimbria-fornix (FF) eliminates cholinergic innervation of the hippocampal formation (HF) as evidenced by a reduction in AChE staining. Fetal septal tissue transplantation restores AChE staining with the same laminar pattern exhibited by the native cholinergic fibers. Entorhinal cortex (ERC) ablations produce alterations in AChE staining; when combined with the FF lesion in transplant animals, an ERC lesion results in an intensification of cholinergic reinnervation which is host specific.

Sixteen young and nine aged F344 rats were subjected to ablation of the FF and ipsilateral ablation of the ERC. Six to eight days following surgery fetal septal neurons (E16-19) were transplanted into the FF cavity. Thirty days after donor tissue grafting, young and aged host animals were studied using AChE histochemistry and retrograde transport (HRP) techniques.

Transplanted fetal septal neurons survived and provided AChE positive fibers to septal and ERC denervated laminae. Ingrowth was more frequently observed in young vs aged adult hosts (69% vs 22% respectively). In the younger hosts, the density and rostro-caudal extent of transplant fiber ingrowth was greater than in the aged hosts. Injections of HRP into the reinnervated zones of the HF resulted in neuronal labeling confined to transplanted septal neurons. Our results show that in each host age group transplanted fetal septal cholinergic neurons can direct their afferents into cholinergic denervated lamina. However, relative to young adults, aged hosts are impaired in their ability to provide a milieu favorable for the transplanted fetal neurons. (Supported by NIH grants NS21541, AG05119, NS00444, AG00084 and the V.A. Medical Research Service)

261.2 DECREASE IN SIZE OF ACETYLCHOLINESTERASE-POSITIVE BASAL FOREBRAIN CELL BODIES IN C57B1/6 MICE BETWEEN 7 AND 53 MONTHS OF AGE. J. Hornberger*, D.G. Flood, T.H. McNeill and P.D. Coleman (SPON: R. Joynt). Departments of Anatomy and Neurology, University of Rochester, Rochester, NY 14642.

We have previously reported stability of numbers of acetylcholinesterase-positive basal forebrain neurons in mice between 15 and 53 months. We here extend the finding of stability of cell numbers to 7 month old mice and report that there is, however, a progressive decline in cell size from 7 to 53 months. Seven 7 month-old, 8 fifteen month-old and 5 fifty-three month-old C57B1/6 mice were obtained from the NIA colony at Charles River Breeding Laboratories. The mice were injected with 2 mg/kg diisopropylfluorophosphate (DFP) in sesame oil 4 hours prior to sacrifice. They were anaesthetized and perfused with mixed aldehydes. The brains were removed and Vibratome sections cut at 20 um. Sections were stained for acetylcholinesterase by the method of Karnovsky and Roots (1964) as modified by El Badawi and Schenk (1967). Tetraisopropyl-pyrophosphoramide was included in the incubation medium to inhibit non-specific cholinesterase. Slides were coded and AChE-positive cells were counted and measured in the medial septum, nucleus of the diagonal band (vertical and horizontal bands), magnocellular preoptic nucleus and nucleus basalis of Meynert. Cells were counted in every third section. Cell sizes were determined by tracing outlines of cell bodies onto the X-Y tablet of an Apple II Plus computer, which then computed the areas of neuronal profiles. Neuron numbers did not change with age in any of the regions examined. Mean neuronal area showed a statistically significant decline with age in all regions examined. The average decrease in area from 7 to 53 months ranged from 6% in the medial septum-diagonal band complex to 15% in the nucleus basalis. The decrease in cell size reported here represents a cellular change which is not associated in the near term with cell death, but which may be associated with declines in the synthetic capacities of these cholinergic neurons.

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261.3 AGE-RELATED CHANGES IN ANDROGEN RECEPTOR LEVELS IN RAT CRANIAL NERVE NUCLEI AND MUSCLES. W.H.A. Yu and M.Y. McGinnis*, Department of Anatomy, Mount Sinai School of Medicine of the City University of New York, New York, NY 10029.

In a previous study we have demonstrated the presence of high affinity cytosolic androgen receptors in motor nuclei of the hypoglossal and facial nerves and in the tongue muscles of adult rats (Soc. Neurosci. Abst. 10:901). In the present study we have examined whether cytosolic androgen receptor levels in these tissues change with aging. Young and old male Fischer 344 rats (4 and 20 months of age, respectively) were orchidectomized under anesthesia 2-3 days prior to sacrifice. Tissues from hypoglossal, facial, and cochlear nuclei were excised under a stereomicroscope. Tongue, masseter, and levator ani muscles were removed and dissected free of extraneous tissue. Pituitaries and the combined tissues from hypothalamus, preoptic area, and amygdala (HPA), that have been previously reported to show age-related changes in steroid receptor concentrations, were assayed simultaneously for comparison. The levels of androgen receptors in the cytosol of tissue samples, pooled from 4-5 rats, were determined by an in vitro binding assay, using $^{3}$H-R1881 as ligand, and expressed as fmoles/mg protein. In old rats there was a significant reduction in the number of cytosolic androgen receptors in all the cranial nerve nuclei examined. The most severe decrease occurred in the cochlear nuclei in which the receptor levels were reduced to only one tenth of the values seen in young rats. In the hypoglossal and facial nuclei, the levels of androgen receptors were reduced to one half of the values of young rats. In contrast, there were no age-related changes in the levels of androgen receptors in HPA or in any of the three muscles examined. The pituitaries of old rats showed a 2-fold increase in cytosolic androgen receptorgs compared to young rats. The decrement in androgen receptor concentration in old rats may render neurons in these cranial nerve nuclei less responsive to steroid hormone regulation and may contribute to an age-related decline in function.

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261.4 MORPHOLOGICAL AND NEUROPHYSIOLOGICAL ANALYSES OF NEURONS IN THE NUCLEUS BASALIS OF MEYNERT OF THE CAT. R.L. Lloyd, R.S. Fisher, C.D. Hull, N.A. Buchwald and M.S. Levine. Mental Retardation Research Center, UCLA School of Medicine, 760 Westwood Plaza, Los Angeles, CA 90024.

These studies were designed to assess some of the morphological and neurophysiological characteristics of neurons in the nucleus basalis of Meynert (NbM) in the cat. In the first experiment retrograde labeling methods were used to demonstrate the distribution of NbM neurons projecting to frontal neocortex. Lectin-bound horseradish peroxidase (HRP) was pressure injected into precruciate cortex of 6 cats. In NbM and the globus pallidus (GP) medium- and large-sized neurons were labeled with the HRP indicating that these neurons projected to the neocortex. In both areas neurons had polymorphic somata with an average cross sectional area of 286 $\pm$ 12 $\mu m^2$. There were no differences between somatic areas of retrogradely filled NbM or GP neurons. Pallidocortical neurons were most prevalent ventrally near the rostral half of the anterior commissure. NbM neurons were distributed widely across the medio-lateral and anterior-posterior extent of the nucleus. Neurophysiological experiments were performed on 5 additional adult cats. Extracellular recordings were made from 69 neurons in NbM and GP. Recording sites were chosen on the basis of the anatomical results indicating the location in NbM and GP of neurons projecting to pericruciate cortex. Responses of NbM and GP neurons to electrical stimulation of precruciate gyrus, postcruciate gyrus and caudate nucleus were determined. Most cells responded to stimulation of caudate and cortex. In both NbM and GP 84% (49/58) of the neurons responded to caudate stimulation, 62% (21/34) to precruciate cortical stimulation and 55% (21/38) to postcruciate cortical stimulation. More than half of the responding neurons displayed initially inhibitory response (57% (28/49) for caudate stimulation and 69% (29/42) for cortical stimulation). Proportionately more responses to caudate stimulation were initially excitatory (43% (21/49)) than responses to cortical stimulation (31% (13/42)). NbM or GP neurons that were antidromically activated by cortical stimulation were not encountered frequently. When such neurons were identified average latency to the antidromic spike was about 10 msec. These neurons also responded orthodromically to cortical and caudate stimulation. Analysis of spontaneous firing patterns indicated that most NbM and GP neurons were either rapidly firing (mean interspike intervals <200 msec; 25% (13/52)) or displayed relatively slow rates (mean interspike intervals >1000 msec; 42% (22/52)). These results indicate that NbM neurons project to neocortex and receive inputs from caudate nucleus and pericruciate neocortex. Supported by USPHS Grants AG 01558 and HD 05958.

261.5 NEUROPHYSIOLOGICAL CHANGES IN CAUDATE NUCLEUS NEURONS IN AGED CATS. M.S. Levine, R. L. Lloyd, C.D. Hull, N.A. Buchwald. Mental Retardation Research Center, UCLA School of Medicine, 760 Westwood Plaza, Los Angeles, California 90024.

These studies were designed to assess some of the neurophysiological changes in caudate nucleus (Cd) neurons in cats over 10 yrs of age. Extracellular single unit recordings were made from the head of the Cd in "acutely" prepared locally anesthetized cats. To date, we have recorded and compared data from 110 neurons in 5 aged cats (11-16 yrs) with data from 122 neurons in 7 adult cats (1-3 yrs). The ability of Cd neurons to respond to stimulation of monosynaptic inputs from precruciate cortex (Cx) and substantia nigra (SN) was determined. In adult cats 81% (89/110) of Cd neurons responded to Cx stimulation and 72% (58/81) responded to SN stimulation. In aged cats there was a decrease in the proportion of neurons responding to SN stimulation (63% (44/70)). The proportion of neurons responding to Cx stimulation remained relatively unchanged (79% (65/82)). Types of responses that were evoked in both adult and aged cats were similar and consisted of either excitation (increase in frequency of occurrence of action potentials), excitation followed by inhibition of action potentials or inhibition alone without preceding excitation. The proportion of time each type of response was evoked in the aged animals when either Cx or SN was stimulated was altered. In adult cats Cx stimulation evoked initially excitatory responses 74% of the time (66/89) while in aged cats excitatory responses occurred 63% (41/65) of the time. When SN was stimulated this decrease in initial excitation was greater (65% (38/58) in adults vs 32% (14/44) in aged cats). In all aged animals but not in adults stimulation thresholds were higher for evoking excitatory responses than were thresholds for evoking inhibitory responses. There was a tendency for more shorter latency responses to occur in the aged animals. In order to assess synaptic security the ability of Cd neurons to respond to iterative stimulation was determined. Distributions of the minimum intervals necessary to evoke two excitatory responses were constructed. There was a marked increase in the proportion of longer intervals in the aged animals indicating that the neuron's ability to respond to iterative stimuli was decreased. These findings provide evidence for decreasing excitability and synaptic security in Cd neurons in aged cats. Furthermore, they indicate that there are functional changes in Cd neurons during aging that parallel some of the morphological changes that we have previously described (Levine et al. Neurosc. Abst. 10, 1984, 451). Supported by USPHS Grants AG 01558, HD 05958.

261.6 EFFECTS OF AGE ON PAIRED PULSE INHIBITION AND POTENTIATION IN CA1 OF THE RAT HIPPOCAMPUS. J. F. Ott, B. E. Hunter and D. W. Walker. Dept. of Neuroscience, Univ. of Florida Coll. of Med. and V. A. Medical Center, Gainesville, FL 32610.

This study was conducted as part of an ongoing project to understand the effects of feed forward (FFI) and recurrent inhibition (RI) in the CA1 of the rat hippocampus. To help dissociate FFI from RI effects, paired-pulse stimulation was delivered to CA1 in either orthodromic/orthodromic (O/O) or antidromic/orthodromic (A/O) pairs. To evaluate age effects on O/O and A/O pairs, in vivo experiments were conducted in 10 month old (Y) or 25-27 month old (O) male Fischer 344 (F-344) rats.

Concentric bipolar electrodes were placed in stratum radiatum at the CA1-CA2 border for orthodromic stimulation and in the alveus for antidromic stimulation. Glass micropipettes were positioned in stratum oriens or radiatum to record EPSP or population spike (PS) responses. To allow comparison between animals, percentages of PS maximum or EPSP at PS threshold were used rather than absolute amplitudes. Orthodromic condition pulse intensities of 25% and 75% of the PS maximum were used as were the current intensity at the PS threshold and 25%, 50% and 75% of the EPSP at PS threshold. Antidromic condition current intensities were either 25% and 75% of the antidromic PS maximum. Test pulse current intensities were either 25% of the PS maximum or 50% of EPSP at PS threshold. Interpulse intervals ranged from 20-3000 msec.

Results from F-344 rats are similar to those we obtained from Long Evans (LE) hooded rats (Roger, C.J., et al., Soc. for Neurosci. Abstr. 10:305.7 1984). Little difference between strains was seen with A/O stimulation. However, when using O/O stimulation, the potentiation of the test pulse was less in F-344 rats, particularly at higher conditioning pulse amplitudes. As in LE rats, inhibition produced by A/O stimulation increased with increasing A/O conditioning pulse amplitude. A/O inhibition was slightly greater in Y animals. O/O stimulation produced potentiation that at higher conditioning pulse amplitudes was obscured by an initial inhibition. This inhibition increased in magnitude and duration with increasing conditioning pulse amplitude and was greater in Y animals, particularly at the higher conditioning pulse amplitudes. The potentiation seen with O/O stimulation increased with increasing subthreshold condtioning pulse amplitudes to reach a maximum when the conditioning pulse amplitude was 75% of EPSP at PS threshold. As the conditioning pulse amplitude was increased further, maximum potentiation decreased. This was true for both Y and O animals, however, potentiation was much greater in O animals. Age differences were much more prominent in the O/O than A/O paired pulse series. Preliminary analysis of these data suggest that FFI is more affected by age than is RI.

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261.7 THE HYPERPOLARIZING DENDRITIC GABA RESPONSE OF THE RAT CA1 HIPPOCAMPAL PYRAMIDAL NEURON DECREASES WITH AGE.
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Pressure application of GABA onto the hippocampal CA1 pyramidal cell causes a hyperpolarizing response in the somatic region and a depolarization (D-DEPOL) in the dendritic region which are both apparently mediated by chloride. Recently a dendritic hyperpolarizing response (D-HYPER) which seems to be due to an increase in calcium dependent K conductance ($gK_{Ca}$) has been described (Blaxter et al., Soc. Neurosci. Abstr., Vol 10, Part 1, p. 203, 1984).

We compared the mid stratum radiatum dendritic GABA response in slices taken from young (6-8 months) and old (25-30 months) Fisher-344 rats. Various amounts of GABA ($10^{-2}$M) were pressure applied into the mid stratum radiatum area of the impaled cell. There was no statistical difference in the resting membrane potential or input resistance between old and young neurons. The total duration of the GABA response was similar although old cells showed more variability. The amplitude of the D-HYPER phase was significantly less in the aged cells ($P<0.05$) but the duration was not significantly different. The amplitude of D-DEPOL phase was similar although the responses of the young cells were shorter, probably due to the greater strength of the underlying D-HYPER. This would indicate that the GABA activated $gK_{Ca}$ in the dendrites is lower in aged animals which might be due to an underlying change in the intracellular calcium regulatory processes.

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261.8 NEURONAL INVOLUTION IN FETAL NEOPALLIAL TRANSPLANTS ISOLATED WITHIN PERIPHERAL NERVES OF ADULT RATS.
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Embryonic E12-13 rat neopallium was dissected, mechanically disaggregated, plated on poly-L-lysine coated tissue culture flasks and grown in a modified Eagle's minimal essential medium. After 3 to 7 days these cells were treated with trypsin, washed and centrifuged in preparation for transplantation into isolated segments of transected sciatic nerves of adult Lewis rats. An incision was made in the epineurium and 250,000 to 500,000 cultured progenitor cells were introduced into the isolated nerve segment. To minimize reinnervation of these transplants the severed ends of the transected nerves were tied off and separated from each other. Neuronal transplants were examined by electron microscopy, immunocytochemistry and morphometry at time periods ranging from 1-3 months (Group A) and 11-12 months (Group B) after grafting.

While neurons in Group A grafts survived and differentiated as described previously (Doering and Aguayo, Soc.Neurosci.Abstr.,10: 49,1984), we observed several different changes in long standing neuronal implants in Group B rats. The main alterations consisted of an intense immunostaining of the neuronal somata by RT97, an antibody directed against the 200 kD neurofilament (NF) protein subunit. The same method predominantly stained axons and dendrites of neurons in Group A grafts . Several other features of neuronal involution were evident at the ultrastructural level: nerve cell nuclei were dense and shrunken (decreased nuclear area), ellipsoid in shape (decreased form factor), contained inclusions (filamentous intranuclear rodlets) and were surrounded by a thin cytoplasmic rim. The neuropil contained accumulations of intermediate filaments, degenerating neuronal processes and many dense and multivesicular bodies. Such morphologic abnormalities were not observed in Group A rats, in cerebral cortices from the host animals nor in the brains of 1 year old rats.

These observations suggest that transplants of rat embryonic cortical neurons isolated in peripheral nerves for long time periods undergo certain involutional changes reminiscent of those described in the brains of aged animals (J.E. Johnson and J. Miquel, Mech. of Ageing & Develop. 3:203, 1974; K.R. Brizzee et al., Aging 7:515, 1978; J.P. Brion et al. Acta Neuropathol.58: 107, 1982; MB. T. Buschmann and A. LaVelle, Neurobiol. of Aging 4:197, 1983). The unusual environmental conditions and deficient afferent and efferent connections of these nerve cell grafts may accelerate involutional processes.

261.9 CALCIUM IN THE SPINE APPARATUS OF DENDRITIC SPINES IN THE DENTATE FASCIA VARIES WITH AGE.
K. Cullen-Dockstader* and E. Fifkova (SPON: A.C. Bekoff). Dept. Psych., Univ. Colorado, Boulder, CO 80309.

Using the pyroantimonate precipitation technique, we have demonstrated $Ca^{2+}$ deposits in the spine apparatus (SA) in dendritic spines (Fifkova, et al., Brain Res. 266:163, 1983). The spine apparatus is connected with the smooth endoplasmic reticulum (SER) of the dendrite, and the SER in general is considered to be a calcium sequestering site. The reported experiments were undertaken to study $Ca^{2+}$ precipitates in the SA in relation to age. Male rats (Fischer 344) ages 3, 9, 24, and 30 months (4 animals per age group) were perfused for electron microscopy and treated for $Ca^{2+}$ cytochemistry. Instead of pyroantimonate we have used oxalate as a primary precipitating agent and pyroantimonate as a secondary one to enhance the contrast of the $Ca^{2+}$ oxalate deposits. In order to minimize the possible method-related artifacts, groups of 4 rats (one from each age group) were perfused and treated at the same time. Spines were scored in the middle and distal thirds of the dentate molecular layers since here they receive a homogeneous input from the entorhinal cortex. Spines were scored as to the presence of the SA and the $Ca^{2+}$ deposits there, and the data were expressed as a percentage of the total number of the encountered spines. For each age group 3,000 spines were scored. A steady decline in the presence of deposits in the SA was observed between the 3, 9, and 24 months - 16.7%, 14.4%, and 11.6%, respectively. While this decline was not significant between the 3 and 9 months groups, it was significant between these groups and the 24 months group, respectively. Given that between these ages there was no significant decline in the presence of the SA in spines, these results may indicate a decreased capacity of the spine apparatus to sequester calcium with age, a conclusion which is in line with the suggested age-related alteration of $Ca^{2+}$ homeostasis (Landfield and Pitler, Sci. 226:1089, 1984). However, in the 30 months group, there was a significant increase of spines with deposits in the SA (18.1%) as compared to 24 and 9 months. Such a result could be related to the loss of spines observed in this region (Geinisman, et al., Anat. Rec. 187:586, 1977). Given that in the 30 months group there is also a significant increase of spines with SA, the spine loss, if it were related to the present data, would have to include specifically spines without the SA. However, the presented results could also be explained by a compensatory growth of the remaining spines with SA.

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261.10 Regional Analysis of Rat Brain Proteins During Senescence
J.W. Cosgrove, J.R. Atack, and S.I. Rapoport Lab. of Neuroscience, NIA, NIH, Bethesda, MD 20205

Previous studies in this laboratory have demonstrated that the overall in vitro protein synthetic capacity of extracts of whole brain of male Fischer-344 rats is age-invariant between young (3-4 months) and old (32-34 months) animals (Cosgrove, J.W. and Rapoport, S.I: Soc. Neurosci. Abstracts 10: 131.16, 1984). Thus, there appears to be no major age-related alteration in the ability to synthesize brain proteins in the male Fischer-344 rat. However, it is possible that, where as in vitro synthetic capacity is unaltered during senescence, abnormal proteins accumulate in the brain due to an increase in in vivo translational errors associated with the aging process. These errors would be manifested as alterations in either the molecular weight of proteins due to inaccurate translation, or as changes in the isoelectric point of proteins due to amino acid substitutions resulting from translational errors. Such changes would not be detected in a study of overall protein synthetic capacity. However, use of the two dimensional gel electrophoresis method in conjunction with either standard protein dye or highly sensitive silver stain detection techniques would provide a means of investigating possible increases in translational errors.

We sampled 11 different regions of the central nervous system of the male Fischer-344 rat between 4 and 30 months of age, including several areas which reportedly show significant age-related changes in cell morphology. Our data indicate that the most abundant brain proteins are expressed at approximately equal levels in the 11 brain regions. Furthermore, the expression of these major proteins is age-invariant. These results agree with a previous study which found no major alterations in proteins in the superior cervical ganglion of the male Fischer-344 rat up to 24 months of age (Wilson et al., Gerontology, 24: 426, 1978). In contrast to the major proteins, a number of the less abundant (but still detectable) brain proteins show region-related differences in the level of their expression at 4 months of age. In addition, preliminary results indicate that the level of expression of several of these proteins is altered in relation to age. Interestingly, the altered expression as a function of age is region specific. Thus, a particular brain protein may have a constant level of expression with age in one brain region, but the same protein may display an age-related alteration in the level of expression in another brain region. These data provide no support for the theory that there is a major accumulation of translational errors or of abnormal proteins in the brains of male Fischer-344 rats in relation to age. However, they do indicate that there is region-specific regulation of expression of a number of brain proteins and that during senescence there may be differential control mechanisms which operate in some brain regions but not in others.

261.11 EFFECTS OF AGING ON ATP-DEPENDENT $CA^{2+}$ TRANSPORT IN BRAIN SYNAPTIC MEMBRANES. M. L. Michaelis and E. Le Cluyse*. Center for Biomedical Research, University of Kansas, Lawrence, KS 66046.

Synaptic plasma membranes contain two $Ca^{2+}$ transporting systems which help to maintain low free intraneuronal $Ca^{2+}$ concentrations, a $Na^{+}$-dependent $Ca^{2+}$ antiporter and a ($Ca^{+}$ + $Mg^{2+}$)-ATPase which pumps $Ca^{2+}$ to the extracellular environment. We have recently observed that aging affects the activity of both of these systems in synaptic membranes obtained from Fisher 344 rats. The affinity of the $Na^{+}$-$Ca^{2+}$ antiporter for $Ca^{2+}$ is decreased in aged animals. The $V_{max}$ of the $Ca^{2+}$-activated ATPase was also slightly decreased in 24-vs 8-month old animals. More recently we have been examining the kinetic characteristics of the ATP-driven $Ca^{2+}$-transport in very highly purified synaptic plasma membranes and the influence of aging on this activity. This transport system is dependent on the presence of $Mg^{2+}$ ($K_{0.5} \approx 55$ µm) and requires the hydrolysis of ATP ($K_m \approx 20$ µM), as non-hydrolyzable ATP analogs will not support $Ca^{2+}$ transport. Possible age-dependent alterations in the activity of this pump have been examined in highly purified membranes from the brains of 7-8 and 23-24-month old Fisher 344 rats. Incubations were carried out in a medium containing membrane protein (≈ 20 µg),100 µM ATP, 50 µM $MgCl_2$, and various concentrations of $CaCl_2$ ranging from .05 µM - 1.0 µM; with 0.2 µM CDTA to buffer the final free $Ca^{2+}$, $Mg^{2+}$, and ATP concentrations. Under these conditions we have found small differences in the kinetic characteristics of the $Ca^{2+}$ transport system between the two age groups. Computer fitting of the data to the Michaelis-Menten equation revealed a decrease in the affinity for $Ca^{2+}$ in the 24-month ($K_M$ = 2.6 µM) compared to the 8-month old animals ($K_M$ = 1.8 µM). However, the $V_{max}$ values obtained from the $Ca^{2+}$ curves for the two groups of animals indicated that the aged animals had an increased maximal transport capacity. It is conceivable that some alteration in the composition of the synaptic membranes has led to a decreased affinity for $Ca^{2+}$, but that the overall transport capacity of the synaptic junctional membranes at high $Ca^{2+}$ concentrations is maintained. Studies are underway to probe the possibility that the aging process leads to changes in the membrane lipid composition which then alters the activity of membrane proteins. (Supported by grants Ag-04762 and NS-16364 and the Center for Biomedical Research, University of Kansas.)

261.12 BRAIN CALCIUM-DEPENDENT PROTEASES, NEURONAL STABILITY, AND AGING: AN HYPOTHESIS. M. Baudry, J. Larson* and G. Lynch, Center for the Neurobiology of Learning and Memory, U.C. Irvine, CA 92717 U.S.A.

Current ideas about the causes of brain aging fail to account for three well-known aspects of the phenomenon: (1) aging is associated with neuronal atrophy and degeneration, (2) since maximum life-span varies by orders of magnitude across the mammals, brain aging presumably progresses at different rates in different species, and (3) the neuropathology associated with aging does not occur with equal intensity in all brain regions and cell types.

We now advance a cellular hypothesis that provides at least a partial explanation for these three features of brain aging. Specifically, we suggest that variations in the activity and/or amount of calcium-activated proteases ("calpains") dictate the rate at which pathology develops in brain with age. Calpain exists in two forms, distinguished by their threshold for activation by calcium; both forms of the enzyme are found associated with soluble and membrane fractions in brain. These enzymes degrade certain cytoskeletal proteins as well as proteins that cross-link cytoskeletal elements with each other or the plasma membrane. Several lines of evidence implicate calpains in the breakdown of the cytoskeleton as well as in muscular and neuronal degeneration. Thus these enzymes provide a plausible candidate for the causes of age-related neuropathology. Maximum life-span in mammals is highly correlated with brain size according to the formula: life span = K (brain size)$^{+0.3}$ ; bats provide a singular exception to this equation in that they live much longer than would be predicted from brain size. We have found that soluble calpain activity is negatively correlated with brain size across orders of mammals according to the formula: calpain activity = K (brain size)$^{-0.4}$, and that bats have anomalously low levels of calpain activity. Combining these points, we find that calpain activity is almost linearly (and negatively) related to maximum life span and thus presumably to the rate of brain aging. Finally, recent immunocytochemical experiments have shown that calpain is not evenly distributed throughout the brain and that it is found in high concentrations in a number of areas and cell types known to be at risk in aging (e.g. subiculum, cerebellar Purkinje cells, large neurons in cerebral cortex).

Thus calpains are linked to degeneration, co-vary with aging rate, and show at least some association with the loci of age-related neuropathology. We propose that calpain activity determines the pace at which cytoskeletal and structural elements of brain cells are broken down and replaced, and that this process, possibly coupled with progressive deterioration of intracellular calcium buffering, is responsible for the gradual appearance of irreversible damage over the course of aging.

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261.13 VESICLE MOBILIZATION AND DEPLETION DURING FREQUENCY POTENTIATION AND DEPRESSION IN THE HIPPOCAMPUS OF AGED AND YOUNG RATS. M.D. Applegate* and P.W. Landfield, Dept. of Physiol. & Pharmacol., Bowman Gray Sch. of Medicine, Winston-Salem, NC 27103.

Although studies in peripheral and invertebrate systems suggest that vesicle mobilization and depletion are correlated with synaptic potentiation and depression, respectively, it is not clear whether a similar relationship is seen in mammalian brain. Moreover, except for one preliminary study, there have been no investigations of vesicle density and concurrent electrophysiology in a monosynaptic brain system. The present studies were therefore undertaken to determine whether vesicle mobilization and depression are correlated with synaptic potentiation and depression in brain. In addition, since previous studies have found a deficit in the amplitude of potentiation and increased synaptic depression in the hippocampus of aged rats during repetitive stimulation, we conducted experiments on vesicle distribution in both aged and young rats before and during a stimulation train.

Stimulating electrodes were placed in the Schaffer-commissural system of field CA1 in the hippocampus of 13 young and 11 aged rats. Recording electrodes were placed in the somal layer of CA1 and population spike responses were recorded. After control responses were obtained, the Schaffer-commissural fibers were stimulated at 10 Hz and at 150% of the voltage necessary to elicit a population spike. Stimulated animals were intracardially perfused with a mixed aldehyde solution after either 45 sec or 8-9 min of stimulation. Control animals were implanted with electrodes but not stimulated. After standard preparation, electron micrographs were shot according to a programmed sequence throughout a strictly defined zone in the region of densest Schaffer-commissural termination on CA1 apical dendrites. Synapses were analyzed for vesicle density and for density of the local vesicle population (LVP - vesicles within the region adjacent to the active synaptic zone).

In both young and aged rats 45 sec stimulation increased the proportion of vesicles near the synapse, while 8-9 min of stimulation was associated with depletion of vesicles. The degree of potentiation or depression of the population spike was correlated with the degree of vesicle redistribution to the active zone or with the degree of depletion, respectively, across individual animals. As a group, aged rats exhibited significantly less mobilization (redistribution) of vesicles at 45 sec and greater depletion after 8-9 min of stimulation.

The results indicate that the relationship between synaptic structure and physiology seen in central systems is similar to that seen in peripheral systems, and that the age-related deficit in hippocampal synaptic physiology may be related to reduced mobilization and/or recycling of synaptic vesicles. (Supported by AG 04542)

261.14 AGE-RELATED CHANGES IN CHOLINERGIC ENZYMES AND MUSCARINIC BINDING SITES IN RAT BRAIN. J.E. Springer, R. Loy, and M. Tayrien*. Dept. of Anatomy, University of Rochester Medical Center, Rochester, NY. 14642.

In the animal and human aging literature there is conflicting evidence with respect to age-related changes in cholinergic muscarinic receptor binding sites. In the present study we used quantitative receptor autoradiography and radiochemical assays to measure possible age-related changes in cholinergic muscarinic binding and choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) activities in young and aged rat brain. A total of 9 young (12 mo) and 9 aged (40 mo) rats were decapitated and the brains bisected. For assay of enzyme activities, six brain tissues were dissected from one hemisphere of each brain: nucleus basalis, medial septum/diagonal band, entorhinal and frontal cortex, dentate gyrus, and Ammon's horn/subiculum. Tissues were homogenized in 10 ml cold water/gm wet weight and triplicate samples assayed for ChAT, using (14C)-acetyl CoA, and for AChE using (14C)-acetylcholine chloride. For autoradiographic analysis, adjacent 20 um sections were preincubated in buffer, and then incubated in 2.0nM [3H]QNB (S.A. 77 Ci/mM), with or without 1 uM atropine, at 25C for 90 min. Sections were briefly washed and rapidly dried prior to exposure for 6 days to LKB Ultrofilm. Following developing, autoradiographs were analyzed using the ARIA program developed for densitometric analysis with the Nikon Magiscan. Regional specific binding was determined for the subiculum, CA1, CA3, dentate gyrus and hilus subregions of the hippocampal formation. Relative optical densities (normalized rank order) was also determined for these regions relative to whole hippocampus.

As reported by others, we find a drop in both ChAT (38%) and AChE (28%) in the septum, and a 46% drop in ChAT activity in the nucleus basalis of aged rats. Activity of both enzymes in frontal and entorhinal cortex and dentate gyrus is equivalent in young and aged brains. By contrast with dentate gyrus, the activities in Ammon's horn/subiculum of ChAT and AChE are 24% and 38% lower, respectively in the aged brains. At all dorsal hippocampal levels in the autoradiographic analysis, specific binding is no different in the aged and control animals, and is relatively constant through the dorsal levels measured. However, at the more temporal levels of the hippocampus, specific binding is higher in the dentate gyrus (25%), CA1 (27%), and subiculum (40%) in the aged brains. These findings indicate that in aged rat hippocampus, there may exist a compensatory muscarinic receptor mechanism which is responding to changes in cholinergic activity, especially in Ammon's horn and subicular regions.

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261.15 AGE-CORRELATED DENDRITIC CHANGES IN MEDIUM SPINY STRIATAL NEURONS OF THE C57BL/6NNIA MOUSE. T.H. McNeill, L. Koek*, S. Brown*, and J. Rafols. Dept. of Neurology, Univ. of Rochester, Rochester, NY 14642; Dept. of Anatomy, Wayne State Univ., Detroit, MI 48201.

Alterations in neurotransmitter systems of the basal ganglia have been postulated to contribute to a disruption of motor function and balance associated with advancing age. Previous biochemical studies in rodent and human brain have reported age-related declines in a number of biologically active substances in the striatum and the substantia nigra, decreases in the number of dopaminergic and cholinergic binding sites in the striatum, and age-correlated morphological changes in dopaminergic neurons of the midbrain. Since it is known that medium spiny neurons (MS) are a principal target population for nigrostriatal dopamine fibers, we undertook a study to examine the question as to whether striatal MS target neurons undergo compensatory dendritic growth or regression with age in response to possible degeneration of neighboring and/or afferent projecting neurons.

For our study, five mouse brains from each of six age groups (3, 6, 10, 20, 25 and 30 mos) were prepared by the Golgi Cox method. Qualitative examination of the striatum showed that MS neurons had oval shaped cell bodies with several primary dendrites emerging from their soma. Primary dendrites lack spines with the density of the spines reaching a maximum on terminal or next to terminal segments. In general, MS neurons in the caudal striatum had larger dendritic profiles than MS neurons at a more rostral level. Data collection at both a rostral and caudal anatomical level of the striatum show that while there was regression in the dendritic processes of some MS neurons, dendrites of other MS neurons remain unchanged or enlarged. Quantitative examination of dendritic profiles showed that between 3 and 10 months of age, there was an overall loss of total dendritic length in MS neurons, primarily in terminal segments, followed by an increase in dendritic length between 10 and 30 months of age. Total dendritic length in aged mice (30 mos) was not statistically different from young adults (3 or 3 mos). Of interest is that the onset of dendritic "growth" at 10 months is paralleled by the initialization of morphological changes in DA neurons of the SN. These data suggest that while there is no comprehensive change in overall dendritic length or total number of dendritic segments in MS neurons between young and old mice, MS neurons of the striatum represent a dynamic population of growing and regressing cells. Our data also support the concept that with normal aging there are two populations of MS neurons in the striatum. One population is characterized by shrinking dendritic profiles and may be dying, while the other group of surviving neurons show normal or even expanding dendrites with age.

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261.16 DENDRITES OF THE SUBICULUM IN HUMAN AGING AND ALZHEIMER'S DISEASE. D.G. Flood, M.A. Tovey* and P.D. Coleman. Depts. of Neurology and Anatomy, Univ. of Rochester Sch. of Med. & Dent., Rochester, NY 14642.

In addition to studying dendrites of layer II pyramidal neurons of parahippocampal gyrus, dentate gyrus granule cells, and hippocampal pyramidal cells in normal human aging and in Alzheimer's disease, we have extended our studies to the subiculum, the major source of efferents from the hippocampus. From over ninety cases obtained at autopsy, five subjects were assigned to each of the following four groups: 1) normal middle-aged adults (ave. age = 51 yr.), 2) normal old adults (ave. age 73 yr.), 3) normal very old adults (ave. age = 90 yr.), and 4) senile dementia of the Alzheimer's type (ave. age = 76 yr.). All cases were free from significant neurological or psychiatric disorders, except the cases in the latter group had a well-documented history of late stage Alzheimer's disease. All cases were also free from significant neuropathology at autopsy, except that cases in the latter group had abundant senile plaques and/or neurofibrillary tangles in the hippocampus and adjacent neocortex. Pyramidal neurons of the superficial layer of the subiculum were randomly selected for analysis from coded 200 um thick sections of tissue prepared by the Golgi Cox method. The groups did not differ significantly in post-mortem time which averaged 12.8 hrs. Preliminary data suggest that unlike previous findings in other regions of the hippocampus which showed age-related dendritic growth, the neurons in the subiculum show age-related decline in both lengths and numbers of segments. Cases with Alzheimer's disease could be grouped into two categories on the basis of the dendritic measures. The first group showed marked declines in dendritic extent, particularly in the apical dendritic tree. The second group of Alzheimer's cases had dendritic measures which were similar to normals of the same age. Supported by NIH grants AG 02680, AG 01121 and AG 03644, and by the Univ. of Rochester Center on Aging.

261.17 A COMBINED GOLGI HIGH VOLTAGE-E.M. STUDY OF DENDRITE TIPS IN OLFACTORY BULB IN AGING SPRAGUE-DAWLEY RAT. A.A. Carboni, Jr.*, M. del Cerro and P.D. Coleman (SPON: J. Albano). Department of Anatomy, University of Rochester, Rochester, NY 14642.

A variety of studies have described region, species, and age-specific growth of dendrites in the brains of aging animals, including human. Usually, this is reported as growth and, in some cases, branching of terminal dendrites. An important question arising from these observations is whether the newly formed dendritic surface at and near the tips of terminal dendrites forms synaptic contact with the surrounding neuropil. Hinds and McNelly (1977) described growth of dendritic material of the olfactory bulb mitral cells of aged Sprague-Dawley rats. We conducted a Golgi high voltage-e.m. study of the same cell type in the same strain to determine whether the tips of growing mitral cell dendrites formed synaptic contact with the surrounding neuropil. Olfactory bulbs were prepared by a technique in which neurons are Golgi-impregnated (Valverde, 1970), sectioned at 150 microns and gold toned (Fairen et al., 1977). The sections were then embedded in plastic for dendritic quantification at the light microscopic level. Following this quantification the terminal regions of individual dendritic segments were cut from the 150 micron section, re-embedded and re-sectioned serially at 1 micron. The serial 1 micron sections were then examined with the high voltage electron microscope to determine the density of synapses at the presumed newly formed region near dendritic terminations.

Supported by grant AG 1121 from the National Institute on Aging. Use of the AEI EM7 Mkll 1.2 MV high voltage electron microscope of the New York State Department of Health and the assistance of Dr. M.J. Song is gratefully acknowledged.

261.18 AN INEXPENSIVE PC BASED SYSTEM FOR QUANTIFICATION OF NEURONAL PROCESSES. A. Moyer*, V. Moyer* and P.D. Coleman (SPON: V. Laties). Department of Anatomy, University of Rochester, Rochester, NY 14642.

We have developed a system for quantification of neuronal processes based on an IBM PC computer and a GTCO graphics tablet that is used to quantify dendritic extent in two or three dimensions. In the most simple use of this system for 2-D quantification, a tracing of the dendritic tree of a single cell is placed on the graphics tablet. Calibration information is entered. The cell body may be entered. Dendritic segments are then entered by moving a cursor along the drawn dendrite. The operator signals each branch point and ending to the computer. The operator indicates whether an ending was the result of sectioning. The computer automatically orders dendritic segments in both somatofugal and somatopetal ordering systems and signals unfilled branch points on its display of the dendritic tree as entered by the operator. When analyzing pyramidal neurons, the system provides separate analysis of the basal tree, apical shaft, oblique dendrites and terminal tuft of the apical tree.

Three dimensional quantification requires the addition of: (1) provision for mixing a video image of the microscope field with the computer graphics display and (2) one stepping motor driver with stepping motor attached to the fine focus control of a microscope. The stepping motor driver uses information from the serial port of the computer to determine the desired focus changes. The operator moves a computer-generated cursor display along a dendrite displayed in the mixed video image produced by this system and signals desired moves of the calibrated focusing stepping motor to the computer. Information about these moves is retained by the computer to provide the third dimension. Both the two and the three dimensional systems provide information about segment numbers and lengths as a function of both somatofugal and somatopetal ordering. A Sholl concentric circles (spheres) analysis is also provided.

This system represents a considerable departure from a highly automated system developed previously in this laboratory. However, the greater degree of operator control over the pace of the system makes this new version much more tolerable to the operator. Software and hardware specifications are available.

Supported by grant AG 1121 from the National Institute on Aging.

261.19 ANTIGENIC SPECIFICITY IS NOT ASSOCIATED WITH ANTI-BRAIN ANTIBODIES IN SERUM FROM HUMANS TO 91 YEARS OLD. K. Leary and P.D. Coleman (SPON: R. Emerson). Department of Anatomy, University of Rochester, Rochester, NY 14642.

Age-related increase in brain reactive antibodies (BRA) has been reported in a number of studies. We examined the specificity for brain of BRA in human serum from subjects of varying ages. We obtained serum from 40 individuals from 8 months to 91 years old. BRA were assessed in frozen sections of young, middle aged and old human superior frontal gyrus by an indirect fluorescent antibody technique in which intensity of fluorescence was determined. Reactions were labelled using FITC conjugated anti-human IgG F(ab')2. Prior to primary incubation aliquots of serum from each person were untreated, or preincubated with 2% gamma globulin free human albumin or with brain homogenate. As a fourth treatment 20 randomly chosen serum samples underwent pepsin digestion and dialysis to cleave the F(ab')2 and Fc portions of the IgG molecule. All slides were read using a Nikon Optiphot fluorescence microscope with a digital microphotometer attachment. A dichroic filter in the photometer beam path blocked lipofuscin fluorescence, and a variable diaphragm allowed determination of fluorescence intensity from a small portion of a single neuron or from the entire field. Fluorescence intensities were measured with a 40x objective. All readings were taken in layer II.

Microphotometer data from single cells and from full fields indicated no significant change in serum binding to brain with increasing age regradless of pre-treatment of the serum. Pre-absorption with brain reduced (but did not emliminate) binding more than any of the other pretreatments. The binding remaining after preabsorption with brain appears to be attributable to non-specific binding.

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261.20 RECOVERY OF VISUAL FIELDS IN MONKEYS DURING CHRONIC DOSING WITH METHYL MERCURY. V. Moyer* and P.D. Coleman (SPON: B. Weiss). Department of Anatomy, University of Rochester, Rochester, NY 14642.

We are conducting studies of chronic methyl mercury produced loss of neurons in monkey visual cortex and the dendritic extent of the surviving neighboring neurons. Visual field defects were used as a behavioral measure of the effect of mercury dosing. Eight juvenile (28-33 months old) Macaca fascicularis monkeys were fitted with occluding contact lenses and trained to monocularly fixate a point straight ahead and then detect and reach for a small marshmallow brought slowly into the visual field from the periphery at one of 8 locations around a perimetry apparatus. Visual fields were tested twice a week. When stable visual fields had been obtained for two weeks, chronic dosing with oral methyl mercury in grape juice was started for six randomly selected monkeys. An initial loading dose of 200 ug/kg body weight of methyl mercury was given twice a week until the blood level of total mercury reached approximately 2 ug/ml of blood. This took an average of seven weeks. Thereafter biweekly dosing was adjusted to maintain total mercury levels near 2 ug/ml blood. Blood levels were sampled biweekly.

Dosing and visual field testing continued for over one year. Dosing of two monkeys was stopped during the resulting visual field loss. These two animals showed rapid recovery of visual fields after the cessation of dosing. Visual field loss was expressed as total degrees of field loss summed over the 8 locations tested. There were major individual differences among monkeys in both the timing and the extent of the visual field loss. The onset of the loss came between 2 and 11 weeks after the start of dosing, with the peak loss coming 10 to 19 weeks after the start of dosing. The peak total degrees lost ranged from 50 to 265. During this time all monkeys showed recovery of visual fields to near pre-dosing levels.

This finding of consistent recovery of function during and after chronic administration of methyl mercury to a primate is unusual in that chronic human methyl mercury poisoning is usually considered to produce long-term visual field deficits. Our findings are, however, in agreement with the data of Merigan, et al. (1983) for chronically exposed monkeys.

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## ENDOCRINE CONTROL OF DEVELOPMENT II

262.1 ACTION OF CORTICOIDS UPON DEVELOPMENT OF ELECTRICAL PROPERTIES IN CULTURED CHICH EMBRYOS BRAIN NEURONES. Beatriz-Fuentes Pardo, Leticia Verdugo-Díaz*, Pedro N. Velázquez*, Marta C. Romano*. Deptos. Fisiología e Histología, Facultad de Medicina, División de Investigación, UNAM.70250. CINVESTAV. México, D. F.

Progressive changes in both passive and active electrical properties in cultured chick embryos brain neurones were recorded from the 3th to the 12th day of development. The effects of corticosterone application in unique doses to 24 h old cultured were also studied. The main results are: a) in corticoid pretreated cells the membrane resting potential recorded at 3th or 6th day of culture showed a significant increment with respect to control, b) the resistance, capacitance and time constant were systematically modified along the twelve days of culture, and the corticoids pretreated cells reached earlier the final values of these parameters than control cells, c) in control cultures the height and frequency of spikes reached values similar to adult neurones around the 9th day; corticoid pretreated cells often showed these values around the 6th day of culture, d) microscopical observations of both control and pretreated cells showed no significant differences in somal size of neurones along development. Suggesting that corticoids act upon differentiation of metabolic and enzimatic processes involved in cellular electrical properties.

262.2 EXTRINSIC CONTROL OF SULFATIDE AND CEREBROSIDE SYNTHESIS IN ISOLATED RAT NEONATAL OLIGODENDROCYTES. R.P. Saneto, M.C. Cardwell,* L.H. Rome,* and J. de Vellis, MRRC, UCLA, Los Angeles CA 90024.

The formation and maintenance of myelin by oligodendrocytes has been known for over sixty years. However, what factors control these processes remain largely obscure. Recently we developed a serumless chemically defined medium that induced proliferation of isolated oligodendrocytes (Proc. Natl. Acad. Sci. (USA) May 10 issue, in press). Immunocytochemical detection indicated that the components of the oligodendrocyte defined medium (ODM) insulin, transferrin and pituitary fibroblast growth factor, induced the expression of myelin basic protein without a concomittant increase in galactocerebroside (GC). This suggests that expression of these two myelination-associated events are to some degree separately controlled. When either triiodothyronine ($T_3$) or both hydrocortisone (HC) and dibutryl cyclic AMP (dbcAMP) were added to ODM, the extent and intensity of GC staining per culture increased significantly. Neither HC or dbcAMP added separately had an effect. Hormonal effects on the synthesis of both GC and sulfatide were measured biochemically by incubation with ($^3$H)palmitate. Cultures were labelled for 24 h and incorporation into GC and sulfatide was determined after alkaline methanolysis by TLC with purified standards. After 5 days of exposure to 15 nM $T_3$ or 1 uM HC and 1 mM dbcAMP, the synthesis of both GC and sulfatide was significantly increased. $T_3$ induced an equal effect on both GC and sulfatide, increasing the amount of synthesis 150% over control cultures. Together HC and dbcAMP were found to increase the synthesis of the myelin glycolipids to different degrees; GC synthesis was increased 100% and sulfatide synthesis 130% over control cultures. These data indicate that $T_3$ and HC and dbcAMP together, can stimulate GC and sulfatide synthesis in isolated neonatal oligodendrocytes. (This work was supported by NIH grand HD06576 and Department of Energy Contract DE-AC03-76-00012).

262.3 TESTOSTERONE INCREASES CELL NUMBERS IN ORGANOTYPIC CULTURES OF FETAL MOUSE SPINAL CORD. K.F. Hauser and C.D. Toran-Allerand. Ctr. Reprod. Sci., and Depts. of Neurol., and of Anat. and Cell Biol., Columbia Univ., New York, NY 10032.

Developmental exposure to androgens is responsible for the appearance of a sexually dimorphic androgen receptor-containing motor nucleus in the spinal cord of the male rat (Breedlove and Arnold, Sci., 210: 564, 1980) and mouse (Hauser and Toran-Allerand, unpublished). In order to characterize the developmental role of androgen on androgen receptor containing neurons and to distinguish it from the contributions of the neuromuscular effector unit, we have studied the effect of testosterone on motoneuron numbers in cultures of all lumbo-sacral levels of the spinal cord, using [3H]-thymidine autoradiography. Mice were mated for a single 45 min. period (7:30-8:15 AM) and checked for sperm plugs immediately thereafter (E-0). On E-10, pregnant mice received two, 250uCi i.p. injections of [3H]-thymidine at 8:00 AM and 12:00 PM. On E12, which precedes a normally occuring period of mouse lumbar motoneuron death (Lance-Jones, Dev. Brain Res., 4: 473, 1980), individual spinal cord segments L1-6 and S1 were removed with their attached dorsal root ganglia. Explants were exposed twice weekly to the steroid-deficient, serum containing medium standard for our laboratory, which was additionally supplemented with muscle extract (5%), 9-day chick embryo extract (5%), and NGF (100ng/ml). Two embryos were taken from each pregnant female; explants from 1 embryo were treated with testosterone (20ng/ml), while those from the second served as controls. After 35 days in vitro, explants were fixed in 10% neutral formalin, and 6um paraffin sections were processed for autoradiograpy. Cells with a distinct nucleolus, and 20 or more silver grains (heavily labeled) were counted from every third section at a magnification of X640.

Preliminary counts indicate that testosterone significantly increased the number of heavily labeled cells at all levels using the $t$ statistic for paired observations ($P<0.05$). Autoradiograms of 3 mice injected on E-10 and killed when adults showed that the most heavily labeled cells were motoneurons. The results suggest that, despite the presence of muscle extract, testosterone per se exerted an additional specific neuronotrophic effect on the pre-explantation labeled motoneurons, probably by enhancing cell survival, or (less likely) by enhancing cell differentiation and/or inhibiting further proliferation. Since comparable spinal cord cultures are unable to aromatize androgens to estrogens (Hauser et al., in preparation), these findings suggest that the observed effect occurs throughout the lumbo-sacral cord and is directly mediated in part by testosterone. (Supported by NIH, NIMH, and the Whitehall Foundation).

262.4 AXONAL DEVELOPMENT IN NORMAL AND HYPOTHYROID RAT CEREBELLAR CORTEX. C. Gravel*, N. Leclerc*, A.V. Plioplys* and R. Hawkes* (SPON: R.F. Estable-Puig) Laboratory of Neurobiology, Laval University, Quebec, Canada G1J 1Z4.

We are using monoclonal antibodies to investigate the effects of hypothyroidism on axonal development in rat cerebellar cortex. Two monoclonal antibodies raised against synaptosomal plasma membranes have been used to follow axonal development in an interneuron (basket cell) and a projection neuron (Purkinje cell). MabN210 recognizes the high molecular weight subunit of neurofilaments and gives exclusively axonal staining in the cerebellar cortex. MabMIT-23 recognizes a neuron-specific mitochondrial determinant and stains all adult neuronal classes, including the axons (Hawkes et al. Cell 28: 253, 1982). The immunocytochemical staining patterns of the antibodies were compared on two groups of developing rats, normal controls and a group rendered hypothyroid with 6-n-propyl-2-thiouracil. Animals were studied through the first postnatal month. The expression of the 210Kd antigen is severely suppressed in hypothyroid animals. In normal rats, immunoreactivity first appears at P12 and attains adult levels at P15. By contrast, there is little of no mabN210-immunoreactivity at P15 in hypothyroid animals. By contrast, the levels of MabMit-23 were little affected. MabMIT-23 and mabN210 both reveal a novel feature of Purkinje cell axonal development: the transient presence of focal axonal swellings. Swellings are seen in both normal and hypothyroid animals but the timecourse is protracted in the latter group. [Supported by grants from MRC Canada and Fonds de recherche en paralysie cérébrale].

262.5 **NEONATAL HYPOTHYROIDISM PREVENTS NATURALLY OCCURRING CELL DEATH IN THE SUPERIOR CERVICAL GANGLION OF THE RAT.** P. Beaston-Wimmer* and A.J. Smolen (SPON: T.J. Cunningham). Dept. of Anatomy, Med. Coll. of Pa./E.P.P.I. Division, Philadelphia, PA. 19129.

Ganglion neurons in the rat superior cervical sympathetic ganglion (SCG) undergo naturally occurring death during the first two postnatal weeks, resulting in the loss of approximately one third of the neurons present at birth. It is generally thought that regulation of neuronal survival during this period depends exclusively on interactions of the developing neurons with their targets, and in the case of sympathetic neurons, with the well-defined target trophic substance, nerve growth factor. However, recent studies have shown that the factors which regulate neuronal survival in vivo are more complex than was previously assumed. For example, it is now known that when neonatal rats are exposed to increased levels of circulating gonadal steroids during the first two postnatal weeks, most of the naturally occurring neuronal death is prevented. Even the normal differences in levels of circulating steroids in neonatal male and female rats is sufficient to result in reduced neuron death in males.

We have recently begun to investigate the importance of thyroid hormone in the development of the sympathetic nervous system. In several regions of the nervous system, abnormal levels of thyroid hormone during the neonatal critical period are known to alter various aspects of development. In the sympathetic nervous system, experimental hyperthyroidism was shown to accelerate maturation of neurotransmission temporarily, while hypothyroidism delayed maturation. In the present study, we produced experimental hypothyroidism by treating newborn rats with daily subcutaneous injections of propylthiouracil (PTU). Littermates received injections of vehicle only. The injection regimen was continued until postnatal day 15, which marks the end of the period of naturally occurring neuron death. In vehicle injected male rats, there were 27,400 $\pm$ 2,011 neurons in the SCG, compared with 33,800 $\pm$ 1,830 in PTU treated animals. Thus, PTU treated rats had 23% more neurons in their SCGs than did control rats. One possible explanation for this finding is that experimental hypothyroidism delays the onset of neuron death. Alternatively, there may be interaction between thyroid hormone and the gonadal steroids. Further experiments are needed to examine these hypotheses.

262.6 ENHANCED MYELINATION OF RAT BRAIN FOLLOWING ADRENALECTOMY. V.L. Friedrich, Jr., K.R. Fairman and J.S. Meyer. Dept. of Biobehavioral Sci., Univ. of Connecticut, Storrs, CT 06268 and Dept. of Psychology, Univ. of Massachusetts, Amherst, MA 01003.

Removal of the adrenal glands from rat pups causes increases in brain weight and in brain content of myelin, isolated by subcellular factionation, and of the myelin-related enzyme 2':3'-cyclic nucleotide phosphohydrolase (Meyer and Fairman, Dev. Brain Res. 17:1-9). The present study was undertaken to verify and characterize the increased myelin content morphometrically.

The adrenal glands of five Sprague-Dawley rat pups were surgically removed at 11 days after birth; five controls were sham operated (laparotomy without adrenalectomy). Animals were sacrificed at 63 days after birth and processed for electron microscopy. Optic nerve was sectioned transverse to its axis, and corpus callosum was cut sagittally, at the midline. Myelin area fraction was determined by point hit counting of randomly placed electron micrographs, and total myelin as the product of myelin area fraction and tract cross sectional area. The number of lamellae and circumference of randomly selected myelinated axons were also determined.

Multivariate analysis of variance showed a significant overall effect of adrenalectomy ($p=.008$), with changes in myelin per tract cross section ($p=.005$), tract area ($p=.008$) and number of lamellae per myelinated axon ($p=.001$).

The total area of myelin per whole tract cross section was increased 24% in the optic nerve and 14% in the corpus callosum of adrenalectomized animals. The number of lamellae per myelinated axon was increased 10% in both tracts. By contrast, the myelin area fraction was unaffected by adrenalectomy.

Regression analysis relating the number of myelin lamellae to axon circumference showed no effect of adrenalectomy, suggesting that the increased number of lamellae in the myelin sheaths of adrenalectomized animals may be caused by an increase in the size of the axons. Analysis of variance also suggested ($p=.07$) increased size of myelinated axons after adrenalectomy.

The present results are consistent with our previous studies and indicate that adrenalectomy increases the amount of myelin, defined morphologically, in fiber tracts of brain. This effect is expressed at least in part as an increase in the thickness of individual myelin sheaths, a change which may reflect increased axonal size. While the mechanisms of action and cellular targets remain to be determined, these results support the notion that adrenal hormones regulate neural development and that early removal of the adrenal glands stimulates the formation of myelin.

Supported by NIH grant NS-17278 and NSF grant BNS-8118073.

262.7 EARLY ADRENALECTOMY PROMOTES BRAIN CELL PROLIFERATION IN RATS. R. Yehuda*, K.R. Fairman*, and J.S. Meyer. Division of Neuroscience and Behavior, Department of Psychology, University of Massachusetts, Amherst, MA 01003

Previous studies in our laboratory have shown that adrenalectomizing rats on day 11 of life leads to an enhancement of subsequent brain growth which is due to at least 2 factors: increased myelination and elevated brain DNA content. The present experiments were designed to determine whether brain cell proliferation is stimulated following early adrenalectomy, and more specifically to assess the time course, regional specificity, and hormonal reversibility of such stimulation. Rats were either adrenalectomized or sham-operated on day 11 postnatal. Two, 7, or 14 days following surgery, subjects were injected s.c. with ($^3$H)thymidine and then sacrificed 1 h later. Brains were removed and dissected into cerebral cortex, hippocampus, midbrain-diencephalon, and cerebellum. DNA in each sample was extracted, assayed, and counted to determine thymidine incorporation as an index of mitotic activity. Increases in DNA labeling (specific activity) were observed in all brain areas of ADX animals, the effect being similar at each time point. To correct for possible group differences in availability of the labeled precursor, results were also expressed in terms of specific activity divided by the radioactivity in the acid-soluble fraction (i.e., relative specific radioactivity). Adrenalectomy also resulted in significant changes in this measure. The effect of glucocorticoid replacement on the rate of cell proliferation was examined in a second experiment in which pups were implanted with Alzet osmotic minipumps (1 ul/h pumping rate) containing either 1.25 or 2.5 mg/ml corticosterone sodium succinate or saline vehicle. One week of corticosterone treatment reversed the effects of adrenalectomy on both specific and relative specific activity in all brain regions and in a dose-dependent manner. We are currently investigating the effects of adrenalectomy on thymidine kinase activity as an independent measure of cell division. Overall, these results indicate that day-11 adrenalectomy leads to a prolonged stimulation of brain cell proliferation in areas where cell formation at this time is exclusively glial (i.e., cerebral cortex and midbrain-diencephalon) as well as in areas where postnatal neurogenesis is known to occur (cerebellum and hippocampus). We hypothesize that this stimulation results from the removal of a tonic inhibitory influence exerted by circulating glucocorticoids in the normal intact animal.

This research was supported by NSF grant BNS-8118073, BRSG grant RR-07048, and a Healey Endowment Award from the University of Massachusetts.

262.8 EFFECTS OF ADRENALECTOMY ON REGIONAL CNS DEVELOPMENT. T. L. Thomas* and L. D. Devenport (SPON: J. T. Braggio). Department of Psychology, University of Oklahoma, Norman, OK 73019.

Adrenalectomy (ADX) increases gross brain weight (Devenport, 1979) by removing endogenous glucocorticoids, not mineralocorticoids or medullary hormones (Devenport & Devenport, in press). This study sought to determine if the brain growth is general or limited to specific regions.

Thirty day old female rats were bilaterally ADX or sham operated under ether anesthesia. Following a 24 hr recovery period in heated units, the rats were housed in pairs for approximately 7 days, then housed individually. They were sacrificed after a total of 45 post-operative days by sodium pentobarbital overdose. Following perfusion with "instant processing" formalin, brains were removed, prepared uniformly, and stored in 10% buffered formalin for 24 hr. After coding for blind analysis forebrains were weighed and frozen in tissue-tek frozen embedding medium. Sections taken for this study conformed to selected representations found in Paxinos & Watson (1982). These hydrated coronal and saggital samples were slide mounted with coverslip and enlarged with a projection microscope for measurement.

Of the variety of structures and areas examined, the parietal and occipital cortices exhibited significant thickening, and the thalamus underwent significant widening in the ADX rats. No other areas or fiber tracts were significantly differentiated across groups. We also observed a trend toward diminished within-group variation in structure size in ADXs. This trend achieved statistical significance in the case of thalamus (-4.3mm Bregma) and septal area (-0.3mm Bregma) width.

The results indicate that the brain growth stimulated by ADX is regionally specific. Only a small trend for general growth was observed. Instead, the major effect was upon thalamus and neocortex. Decreased within-group variability was observed and adds to the commonalities between ADX- and environmental enrichment-induced brain growth (Cummins, et al, 1977). Both procedures seem to decrease the variability of brain size and to increase cortical thickness. While the fine structural basis of the ADX-induced growth is presently under investigation, our failure to find significant thickening of heavily myelinated fiber tracts suggests that it is not myelination, at least of our selected structures, that lies at the basis of the growth.

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262.9 IS MEDIAL PREOPTIC OPIATE RECEPTOR ONTOGENY ANDROGEN OR ESTROGEN DEPENDENT? R. P. Hammer, Jr. Dept. of Anatomy and Reproductive Biology, University of Hawaii School of Medicine, Honolulu, Hawaii 96822.

The opiate receptor content of the rat medial preoptic area (MPOA) exhibits sexual dimorphism and varies across the estrous cycle in adults (Hammer, Brain Res., 308: 172). Moreover, a dense concentration of opiate receptors develops in the MPOA shortly after birth in females. However, this early receptor development is prevented in females by daily postnatal testosterone administration, and it is fostered in males by neonatal gonadectomy (Hammer, Brain Res., in press). Since estrogen is present even in neonatal brains due to aromitization of androgen to form estrogen, MPOA opiate receptor ontogeny could be dependent on interaction with either of these steroid hormone subclasses.

To elucidate this interaction, film autoradiographs of [$^3$H]naloxone binding in brain sections from five-day old (postnatal day 6 [D6]) male and female rats were examined. Males had been injected subcutaneously on postnatal day 2 with 100 µg of the estrogen antagonist, tamoxifen, in 50 µl sesame oil. Females received daily injections of 250 µg of dihydrotestosterone (DHT), a non-aromatizable androgen compound, in 25 µl sesame oil. Control animals received the same doses of vehicle on the same schedule as experimental animals. Oil-treated females had significantly greater MPOA opiate receptor labeling than all other groups. A dense concentration of MPOA opiate receptors developed in oil-treated females, but not in oil-treated males. Also, DHT prevented the formation of a dense MPOA opiate receptor concentration in females and tamoxifen did not allow its formation in males.

These results further substantiate the importance of sex hormones on MPOA opiate receptor ontogeny during the early, postnatal critical period. When administered during this period, androgen alone is enough to prevent MPOA opiate receptor development; furthermore, blockade of estrogen receptors does not alter this androgen-induced effect. Therefore, it would seem that inhibition of MPOA opiate receptor development is a complex phenomenon not dependent solely on estrogen receptor interaction, rather more dependent on the influence of compounds on androgen receptors. Ongoing studies using androgen receptor blockers should confirm the androgen dependence of this effect.

262.10 DETECTION OF A SOMATOMEDIN -C RELATED mRNA IN RAT BRAIN. P.K. Lund*, B.M. Moats-Staats*, M. Hynes, E. Hoyt*, T. Verdoorn*, V.K.M. Han*, A.J. D'Ercole*, M. Jansen*$^{\pm}$, L. Van den Brande*$^{\pm}$ and J.J. Van Wyk*. School of Medicine, University of North Carolina, Chapel Hill, 27514 and Dept. Pediatrics, State University of Utrecht, The Netherlands.(SPON:R.Dingledine)

Somatomedins or insulin-like growth factors (Sm/IGF) are peptide mitogens thought to be involved in fetal and post-natal growth. Recent evidence indicated the presence of somatomedins in mammalian brain ( Binoux et al, FEBS Lett. 124, 178, 1981; Sara et al., Acta Physiol. Scand. 115, 467, 1982;. ) and suggested that human brain contains predominantly IGF-II rather than Sm-C ( Haselbacher et al, PNAS 82, 2153, 1985). We investigated the production of Sm-C encoding mRNAs in fetal and newborn rat brain to determine whether there was de novo synthesis of Sm-C. Poly (A) RNA from16 to 20 day embryonic, newborn,14 day old and adult rats was size fractionated on agarose gels, transferred to nylon membranes and hybridized with a $^{32}$ P labelled cDNA probe encoding human pro-Sm-C (Jansen et al, Nature 306, 609,1983) A mRNA of 1.75 kilobases (kb) was present in brain poly (A) RNA at all time points investigated except adult, and was more abundant in brain from fetal and newborn animals than14 day old animals These data indicate that rat brain synthesizes a mRNA encoding a protein related to Sm-C and that this protein may be implicated in growth and development of rat brain. Soares et al (Nucleic Acids Res. 13, 1119, 1985) detected a 1.75kb mRNA in neonatal rat brain, by hybridization to a short cDNA probe which showed partial homology to 5' untranslated region and signal peptide coding sequences of rat IGF-II mRNA. This mRNA did not hybridize to cDNA sequences encoding the IGF-II sequence. The precise structure of the 1.75 Kb rat brain mRNA remains to be determined by sequencing of cDNAs However, the mRNA appears to share sequences common to human Sm-C and rat IGF-II mRNAs and may represent the rat Sm-C mRNA or a mRNA encoding an as yet uncharacterized growth factor.

262.11 THE EFFECTS OF ACTH AND PREDNISOLONE ON ELECTROSHOCK SEIZURES, DEVELOPMENT, AND BRAIN WATER AND ELECTROLYTE CONTENTS OF NEWBORN RATS. T. Honda, H.S. White, S.Y. Chow, J.W. Kemp, D.M. Woodbury (SPON: P.R. Burgess). Dept. of Physiol. Univ. of Utah, S.L.C., UT 84108.

ACTH and prednisolone (PSL) have been used for the treatment of infantile spasms. Cortisol has been reported to produce biphasic effects on brain excitability which are age-dependent (Vernadakis and Woodbury, J.P.E.T., 139:110, 1963). The present investigation attempts to identify specific biochemical effects of these agents on the brains of neonatal rats that correlate with their anticonvulsant activities.

Neonatal rat pups were administered either 0.9% saline (2.5 ul/gm body wt.), ACTH (0.5 mg/kg, s.c.) or PSL (2.5 mg/kg, s.c.) on postnatal days 0-3, or 4-7, or 8-11. On postnatal day 15, each animal received an electroshock stimulus (50 mA for 0.2 sec at 60 Hz), through corneal electrodes. Their response to this stimulus was graded as follows: 1 (running seizure), 1.5 (running plus opisthotonus), 2 (forelimb clonus). These were averaged and compared with controls that received only saline. On the 16th postnatal day, under ether anesthesia, blood and CSF were collected for determination of electrolytes ($Na^+$, $K^+$ and $Cl^-$), animals were then sacrificed and the brain dissected out. Cerebral cortex, cerebellum and brain stem tissues were analysed for water and electrolyte contents.

Results suggest that brain excitability as measured by electroshock stimulus was decreased by pretreatment of animals with ACTH or PSL on days 0-7, but was increased by delaying treatment until postnatal days 8-11. CNS development as estimated by eye opening appeared to be enhanced by treatment with ACTH or PSL given on days 0-3 or days 4-7. On postnatal day 14, eyes were open in 90% of animals receiving ACTH on days 0-3, and 83% of animals treated with PSL on days 0-3. This compares with 44% in control animals. In those animals treated between days 4-7, eyes were open in 40% of the controls, 75% of ACTH group, and 100% of the PSL group. Cerebellar water content was reduced by treatment with ACTH on days 4-7 and 8-11. Treatment with PSL on days 4-7 reduced the water content of the cerebral cortex, cerebellum and brain stem. In contrast, when treatment with PSL was delayed until 8-11 days, no change in water content was observed. Reductions in brainstem sodium (12%) and chloride (21%) were observed following treatment with PSL between days 0-3. A decrease in chloride (10%) was also observed in the cortex of animals treated with PSL on postnatal days 4-7. In addition, PSL (4-7 days) increased the cerebral cortex potassium content. No change in tissue electrolyte content was observed following treatment with ACTH. These results suggest that treatment with ACTH or PSL early in development is more effective in producing an anticonvulsant effect than if treatment is witheld until later in development. We are presently attempting to correlate the above reported changes in water and electrolyte contents with the activities of the transport enzymes $Na^+/K^+$-ATPase and $HCO_3^-$-ATPase. These data will provide useful information pertaining to the effects of ACTH and PSL on brain excitability and their use in the therapy of infantile spasms.

262.12 POSTNATAL DEVELOPMENT OF 'SMOOTH' AND 'SPINY' LHRH CELLS: EFFECT OF NEONATAL GONADECTOMY. S.Wray and H.Gainer. Laboratory of Neurochemistry and Neuroimmunology, National Institute of Child Health and Human Development, Bethesda, MD 20205.

Two distinct morphological luteinizing hormone releasing hormone (LHRH) cell types have been previously identified in male and female rats; smooth LHRH cells (sLHRH) and irregular LHRH cells with spinelike processes (iLHRH). The number of iLHRH cells increases postnatally, stabilizing shortly after puberty, suggesting that new synaptic inputs to LHRH cells may be important for the timing and maintenance of reproductive maturation. Gonadal steroids affect LHRH secretion and are critical for reproductive function. This study examines whether gonadal steroids influence the development of smooth and/or irregular LHRH cell types.

One to 2 days post-parturition, 3 male and 3 female Sprague-Dawley rat pups were bilaterally gonadectomized. After surgery, animals were placed under a warming lamp until active and then returned to their mother and left undisturbed for 8 days, after which body weights of gonadectomized and control pups were recorded daily. All animals showed a steady gain in body weight. Pups were weaned and separated according to genotypic sex on postnatal (pn) day 25. At pn days 50-71, animals were anesthetized and perfused with saline followed by 4% paraformaldehyde/0.2% picric acid in 0.1M sodium cacodylate buffer (pH 7.4) with or without 0.15% glutaraldehyde. Consecutive vibratome sections (50 um) were taken from the olfactory peduncles caudally through the median eminence. Tissue sections were blocked in normal goat serum (10%,1hr) and immunocytochemically processed for LHRH. For light microscopy, sections were incubated in LR-5 antiserum (Gift from Drs. Benoit and Guilleman) diluted 1:20,000 in 0.4% triton X for 24 hrs., followed by an avidin-biotin-horseradish peroxidase procedure (Vectastain). For electron microscopy, sections were reacted as above, but no triton X was used. After visualization of LHRH neurons, sections were rinsed in buffer, trimmed, placed in 1% $OsO_4$ (1/2- 1 hr at room temp), dehydrated and embedded in LR White. Silver sections were placed on Formvar-coated slot grids and counterstained with uranyl acetate and lead citrate.

The total LHRH cell number (between 1050-1300 immunoreactive LHRH cells), and distribution of LHRH cells and fibers were unaffected by neonatal gonadectomy. The percentages of sLHRH cells and iLHRH cells in control and gonadectomized animals were identical. Therefore, removal of gonadal steroids in the neonate did not prevent the pn development of the LHRH cell population. Possible alterations in synaptic inputs to sLHRH cells and iLHRH cells after neonatal gonadectomy are currently being examined by immunocytochemical methods at the electron-microscopic level.

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## REGIONAL LOCALIZATION OF RECEPTORS AND TRANSMITTERS II

263.1 THE ONTOGENETIC DEVELOPMENT OF 5-$HT_1$ RECEPTORS IN THE RAT BRAIN: AN AUTORADIOGRAPHICAL STUDY. T. Glaser*, M. Rath*, J. Traber*, K. Zilles*† and A. Schleicher*†, (SPON: F.-K. Pierau) Department of Neurobiology, Troponwerke, D-5000 Cologne 80, FRG and †Anatomical Institute, Univ. of Cologne, D-5000 Cologne 80, FRG.

The densities of serotonin$_1$ (5-$HT_1$) binding sites in various cortical regions of the rat brain at various stages of postnatal development were studied by means of quantitative autoradiography.

In all regions studied, the increase in binding site density between the first postnatal day and adult age was sigmoidal. This increase in the various regions showed a marked heterochrony. Binding sites developed earlier in neocortex than in allocortex. 50% of the adult binding site density was reached in the following order: motor cortex (day 9) > primary somatosensory cortex (d 10) > prefrontal cortex (d 12) > hippocampal formation (d 14-20). It is very interesting that there was also a heterochrony found within the hippocampal formation, with the fascia dentata having the fastet and the $CA_1$ region the slowest increase in receptor density. Adult values of recognition site numbers in the various cortical areas were also reached at different ages. The highest number of 5-$HT_1$ binding sites was found in the dorsal subiculum, the lowest in the primary somatosensory cortex. Radioligand saturation experiments using whole brain membranes corroborated the autoradiographical findings.

The results demonstrate a systematic developmental direction from iso - to allocortical areas.

263.2 MULTIPLE SEROTONIN RECEPTORS IN THE HUMAN BRAIN: CHARACTERIZATION, MAPPING AND EFFECTS OF DISEASE. J.M. Palacios[1], A. Pazos[1*], D. Hoyer[1*] and A. Probst[2*]. [1]Preclinical Research, Sandoz Ltd. and [2]Dept. Pathology, University of Basle, Basle Switzerland.

We have studied the pharmacological properties and the anatomical localization of the proposed subtypes of 5-HT receptors (5$HT_{1A,1B,1C}$ and 5-$HT_2$) by both membrane binding assays and quantitative autoradiographic studies, using labeled ligands selective for the different binding sites. These ligands included $^3$H-5-HT (5-$HT_1$ sites), $^3$H-8-hydroxy-2-(NN-dipropylamino)-tetraline (8-OH-DPAT) (5-$HT_{1A}$), $^{125}$I-cyanopindolol (5-$HT_{1B}$), $^3$H-LSD (5-$HT_{1A,1C}$, 5-$HT_2$), $^3$H-mesulergine (5-$HT_{1C}$) and $^3$H-ketanserin (5-$HT_2$).

Both, similarities and differences were found in the pharmacology and distribution of the 5-HT sites in the human brain, when compared to the rat brain. The affinities of different compounds for 5-$HT_{1A}$ sites in the human were similar to those previously found in the rat. The anatomical distribution of these 5-$HT_{1A}$ sites in the human brain was also similar, in general terms, to that seen in the rat, the hippocampus, raphé nuclei, amygdaloid complex and several cortical fields being enriched in these sites. However, some differences were found; for instance, the dentate gyrus, which is the part of the hippocampal formation presenting the highest density of 5-$HT_{1A}$ binding sites in the rat, only contained a moderate density of specific binding in the human. The 5-$HT_{1C}$ receptor sites in the human brain presented a pharmacological profile similar to that found in the rat, although a certain decrease in the affinity of all the drugs tested was observed. Like in the rat and pig, the human choroid plexuses contained high density of 5-$HT_{1C}$ sites. The species differences were much more marked in the case of 5-$HT_{1B}$ and 5-$HT_2$ sites, where important changes in the pharmacological properties between rat and human brain were found. For example, $^3$H-mesulergine, which label 5-$HT_2$ receptors in the rat, did not recognize them in the human brain. Other substances showing high affinity for 5-$HT_2$ sites in the rat brain were less effective when tested in the human. Regarding the anatomical distribution of these 5-$HT_2$ receptors in the human brain, the different cortical fields, including the frontal, temporal, parietal, visual, cingulate and entorhinal areas, were the most enriched regions. The human mammillary bodies also presented relevant densities of 5-$HT_2$ sites.

Finally, a comparation between the distribution and pharmacology of the different 5-HT sites in the normal brain and that in cases of senile dementia Alzheimer type and Parkinson's disease will be presented.

263.3 **QUANTITATIVE AUTORADIOGRAPHIC LOCALIZATION OF NEUROTRANSMITTER BINDING SITES IN AMYOTROPHIC LATERAL SCLEROSIS.** S. Manaker, C.H. Rhodes, C.M. Wieczorek* and A. Winokur. Departments of Psychiatry, Pharmacology and Pathology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

We used quantitative autoradiography to localize neurotransmitter receptors and uptake sites in spinal cords from three non-neurological controls and three patients with ALS. We examined the densities of TRH receptors with 10 nM ($^3$H)-methyl-TRH, β-adrenergic receptors with 150 pM ($^{125}$I)-iodopindolol, norepinephrine uptake sites with 2 nM ($^3$H)-desmethylimipramine, muscarinic receptors with 2 nM ($^3$H)-quinuclidinyl benzilate, and choline uptake sites with 40 nM ($^3$H)-hemicholinium-3. Sequential sections from cervical, thoracic, lumbar and sacral spinal cord were used for total and non-specific binding for each ligand.

Large decreases in the density of TRH receptors were found in Lamina IX of ALS spinal cords. In thoracic spinal cord, the density of TRH receptors was reduced by 80% (ALS, 16 ± 6 fmol/mg P; control, 82 ± 15 fmol/mg P). Smaller reductions were noted in cervical (ALS, 28 ± 11 fmol/mg P; control, 58 ± 11 fmol/mg P) and lumbar (ALS, 14 ± 10 fmol/mg P; control, 43 ± 6 fmol/mg P) spinal cords. In contrast to TRH receptors, muscarinic receptor densities appeared to be diminished throughout the ventral horn of ALS spinal cord. In Lamina IX, muscarinic receptors were decreased in concentration by 56% in the cervical cord (ALS, 217 ± 26 fmol/mg P; control, 495 ± 63 fmol/mg P) and 68% in the lumbar cord (ALS, 200 ± 30 fmol/mg P; control, 626 ± 116 fmol/mg P). Reductions of 66% and 67% were observed in sacral and thoracic ALS cords, respectively. In the remaining laminae of the ventral horn, decreases in muscarinic receptor densities ranged from only 30% in the thoracic cord (ALS, 161 ± 18 fmol/mg P; control, 229 ± 12 fmol/mg P) to over 60% in sacral cord (ALS, 145 ± 43 fmol/mg P; control, 360 ± 71 fmol/mg P). No alterations in β-adrenergic receptors, noradrenergic uptake sites, or choline uptake sites were noted in adjacent sections of ALS spinal cords.

These data replicate some previous findings in the literature. However, this is the first report, to our knowledge, of normal levels of noradrenergic and choline uptake sites in ALS tissues. Since we simultaneously assayed several neurotransmitter binding sites in sequential sections from the same block of tissue, it is clear that in Lamina IX of the ventral horn, specific depletion of some neurotransmitter binding sites occurs in ALS while other binding sites remain intact. That depletion of both TRH receptors and muscarinic receptors occurs in ALS may reflect an aspect of the pathophysiology of the disease, rather than non-specific loss of receptors localized on degenerating motor neurons. (Supported by NIMH RSDA MH00044 and NIH NS18332 to AW, MSTP NIH 5-T32-GM07170 to SM, NIH 2-T32-NS07064 to CHR and NIH NS19597 and NS20006 and fellowships from the Sloan Foundation and the Klingenstein Fund).

263.4 **AUTORADIOGRAPHIC LOCALIZATION OF BETA-ADRENERGIC RECEPTORS IN HUMAN BRAIN.** G. Reznikoff,* S. Manaker, C.H. Rhodes, A. Winokur and T.C. Rainbow. Departments of Psychiatry, Pharmacology and Pathology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Little information is currently available on the localization of noradrenergic systems in the nuclei and subregions of the human central nervous system (CNS). We have employed the technique of quantitative autoradiography to examine the distribution of β-adrenergic receptors in post-mortem human brain. None of our subjects died as a result of psychiatric, neurologic or endocrine disease. The radioligand used, ($^{125}$I)-iodopindolol (IPIN), bound with an appropriate affinity to post-mortem hippocampus ($K_d$ = 93 ± 30 pM). In addition, the potency of l-propranolol ($IC_{50}$ = 1.1 ± 0.7, N = 3) was 50-fold greater than the potency of d-propranolol ($IC_{50}$ = 50.8 ± 19.1, N = 3) at inhibiting ($^{125}$I)-IPIN binding in hippocampus. These pharmacologic data suggest that our autoradiograhic technique detects authentic β-receptors in post-mortem human tissues.

Highest concentrations of β-receptors (> 50 fmol/mg protein) were found within all subfields of the hippocampus, and high to moderate concentrations were observed in the cerebellum. The thalamic nuclei, basal ganglia, midbrain and amygdala contained moderate densities of β-receptors (25-50 fmol/mg P). All areas of the cerebral cortex, including frontal, cingulate, insular and temporal cortices, were also moderately labeled by ($^{125}$I)-IPIN. In addition, the individual layers of the cerebral cortex were homogeneously labeled in moderate densities. The hypothalamus contained low levels of β-receptors (< 25 fmol/mg P), which were comparable to the β-receptor levels noted in white matter.

The autoradiographic distribution of β-receptors differs from the regional β-receptor densities reported from homogenate binding studies, which reported moderate densities of hippocampal β-receptors, probably reflecting dissection artifacts. The dense concentration of β-receptors in the human hippocampus may suggest functions for NE in the human CNS, including effects on neuroendocrine regulation and memory processing. The distribution of β-receptors in human brain also differs from the β-receptor distribution observed in rat brain. For example, the superficial layers of rat cerebral cortex are heavily labeled by ($^{125}$I)-IPIN, with large differences observed between β-receptor densities in different cortical regions (PNAS 81: 1585, 1984). No such variation existed in human brain, suggesting that β-receptors may play a relatively minor role in cortical functions. (Supported by MSTP NIH 5-T32-GM07170 to SM, NIH 2-T32-NS07064 to CHR, NIMH RSDA MH00044 and NIH NS 18332 to AW, and NIH NS19597 and NS20006 and fellowships from the Sloan Foundation and the Klingenstein Fund to TCR).

263.5 **AUTORADIOGRAPHIC LOCALIZATION OF THYROTROPIN-RELEASING HORMONE (TRH) RECEPTORS IN HUMAN BRAIN.** A. Winokur, S. Manaker, A. Eichen,* C.H. Rhodes and T.C. Rainbow. Departments of Psychiatry, Pharmacology and Pathology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Thyrotropin-releasing hormone (TRH) exerts many effects upon central nervous system (CNS) function. We have utilized the technique of quantitative autoradiography to anatomically localize specific receptors for TRH in post-mortem human brain. None of our subjects died as a result of psychiatric, neurologic or endocrine disease. The radioligand used, ($^3$H)-3-methyl-his$^2$-TRH (($^3$H)MeTRH), bound with similar affinity to post-mortem human pituitary gland ($K_d$ = 9.5 ± 1.4 nM) as to post-mortem amygdala ($K_d$ = 9.6 ± 2.0 nM). In addition, unlabeled MeTRH was 10- to 20-fold more potent than TRH at inhibiting ($^3$H)-MeTRH binding. These data suggest that our autoradiographic technique detects authentic TRH receptors in post-mortem human tissues.

An animal model was used to show that post-mortem decay causes little alteration in TRH receptor densities. We quantified TRH receptor concentrations in 18 different structures from the septal and amydgaloid regions of 24-hr post-mortem rat brain, and compared these values to our previously reported (J. Neurosci. 5:167, 1985) concentrations in freshly sacrificed rats. Only small increases and decreases in TRH receptor concentrations were observed, and more importantly, the relative distribution of TRH receptors was maintained ($r_s$ = 0.9195; $p < 0.001$).

Within human brain, high concentrations of TRH receptors (200-400 fmol/mg protein) were localized throughout the amygdala, including the cortical, basal and lateral nuclei, and also within the stratum oriens of the hippocampus. Low levels of TRH receptors (< 50 fmol/mg protein) were localized within the other layers and subfields of the hippocampus, as well as within all areas of cortex, basal ganglia, and diencephalon.

While a few non-hypophysiotropic actions of TRH on human CNS function are known, this distribution of TRH receptors may be consistent with possible sites of action of TRH in human brain. More importantly, the discrete localization of TRH receptors provides a neuroanatomical template for focusing investigations into the effects of TRH on the human CNS. (Supported by NIMH RSDA MH00044 and NIH NS18332 to AW, MSTP NIH 5-T32-GM07170 to SM, NIH 2-T32-NS07064 to CHR and NIH NS19597 and NS20006 and fellowships from the Sloan Foundation and the Klingenstein Fund to TCR.)

263.6 MODULATION OF HYPOTHALAMIC PROGESTIN RECEPTOR LEVELS BY THE NORADRENERGIC SYSTEM IS SPECIFIC TO THE VENTROMEDIAL NUCLEUS. J. Thornton, B. Nock , B. McEwen and H. Feder. Institute of Animal Behavior, Rutgers University, Newark, NJ 07102 and Rockefeller University, New York, NY 10021.

Progesterone is involved in both the induction of sexual behavior and gonadotropin surges in female guinea pigs. Progesterone appears to act through high affinity, specific intracellular receptors which are highly concentrated in the hypothalamus/preoptic area and are induced by estradiol. The noradrenergic system modulates the level of estradiol-induced cytosolic progestin receptors in hypothalamus. Treatment with the α1 antagonists phenoxybenzamine or prazosin or with the dopamine β-hydroxylase inhibitor U-14624 decreases the number of cytosolic progestin receptors (CPRs; Nock et al, Brain Res., 207:371, 1981; Nock and Feder, Brain Res., 310:77, 1984). The present experiment was designed to more precisely localize where within the hypothalamus noradrenergic (NA) function affects cytosol progestin receptors. Ovariectomized adult guinea pigs were given three daily injections of 20μg estradiol-17β benzoate (EB). Twenty four hours later, females were injected s.c. with either 5mg/kg prazosin HCL in 25% propylene glycol vehicle or the vehicle alone. Six hours later, females were anesthetized, perfused with 10% DMSO and brains were removed and frozen. Coronal sections (300μm thick) were cut and areas were dissected by the method of Palkovits (Brain Res., 59: 499, 1973). Cytosols from microdissected areas were assayed for cytosolic progestin receptors with 0.6nM [3H]R5020 (a synthetic progestin) ± 100 fold excess unlabelled R5020. Tissue from 1-3 females was pooled per sample, N=7-16 samples per area. When hypothalamic areas were examined, it appeared that the decrease in estradiol-induced CPRs in whole hypothalamus is due to a selective decrease in the ventromedial nucleus (VMN). Prazosin resulted in VMN CPR levels of 22.4±1.3 fmoles/mg which is significantly decreased compared to the level of 32.2±4.0 fmoles/mg seen in vehicle treated females. Prazosin did not decrease CPR levels in the periventricular area (PVE) of the anterior hypothalamic nucleus (praz=31.2±6.3, veh=24.2±5.4), the PVE of the ventromedial hypothalamus (praz=40.5±10.3, veh=37.9±9.1), the anterior hypothalamic nucleus (praz=12.8±2.2, veh=12.4±1.4) nor the arcuate-median eminence (praz=185.3±12.2, veh=201.2±11.4). Consistent with other reports, prazosin did not significantly decrease CPR levels in any preoptic areas examined. This was true for both the medial preoptic area and the periventricular preoptic area. In sum, the α1 antagonist prazosin caused a selective decrease in estradiol-induced CPRs in the ventromedial nucleus of the hypothalamus. Because lordosis induced by estradiol and progesterone is also blocked by α1 antagonists it is possible that the noradrenergic system acts at the VMN to modulate both CPR number and lordosis responsiveness.

263.7 HIPPOCAMPAL BETA$_1$-ADRENERGIC RECEPTORS: EVIDENCE FOR LOCALIZATION ON DENTATE GRANULE CELLS. J.K. Tayyeb*, D. Lorton and J.N. Davis (SPON: A.D. Roses). VA Med. Ctr. and Depts. of Med. (Neurology) and Pharmacol., Duke Univ., Durham, NC 27705.

$B_1$-adrenergic receptors have a specific topography in the hippocampus, being found in the hilus and molecular layer of the dentate gyrus. The cell type which possesses the $B_1$-receptor is not known. We have hypothesized that these receptors are located on dentate granule cells. To test this hypothesis we have injected colchicine, a selective neurotoxin which preferentially destroys granule cells, into the hippocampus and used quantitative in vitro receptor autoradiography to study alterations in $B_1$-receptor levels.

Colchicine (0.25 ul, of 2.5 ug colchicine in saline) was injected unilaterally into the ventral hippocampus of adult male Sprague-Dawley rats. Two weeks after the injection, the rats were sacrificed and the brains processed for $B_1$-receptor autoradiography using [$^{125}$I]-cyanopindolol and the specific $B_2$ antagonist ICI 118,551. Densitometric readings were made in $CA_1$, $CA_3$, the molecular layer of the dentate gyrus, and the subiculum. The molecular and granule cell layer widths were measured in cresyl violet stained sections.

Relatively high levels of $B_1$-receptors were located in $CA_1$, $CA_3$, the molecular layer of the dentate gyrus and the subiculum on the control side. Colchicine injection resulted in a loss of granule cells in the dentate gyrus without damage to the pyramidal cells. On the injected side, a decrease in density of $B_1$-receptors in the molecular layer and $CA_3$ corresponded to a decrease in molecular and granule cell layer widths across all sections suggesting that dentate granule cells have $B_1$-receptors located on their dendritic and axonal projections. An unexpected decrease in density of $B_1$-receptors was found in $CA_1$ and subiculum and was not associated with the decrease in of granule cell or molecular layer width. We believe the decrease in subicular receptor density to be the result of transynaptic events, but cannot exclude the possibility that colchicine has biochemical effects on subicular cells without producing histological evidence of damage.

(supported by VA, NIH AG00007 and NS06233)

263.8 DETERMINATION OF 12-14 BIOGENIC AMINES IN BRAIN TISSUE DURING A SINGLE HPLC RUN OF 20 MINUTES WITHOUT PRIOR CLEAN-UP OF SAMPLES M.G. Hadfield and N. Narasimhachari. Neurochemistry Research Laboratory, Dept. of Pathology and Psychopharmacology Research Laboratory, Dept. of Psychiatry, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298

High performance liquid chromatography with electrochemical detection (HPLC-Ec) is now the method of choice for quantitating biogenic amines and their metabolites in brain tissues. But in many solvent systems (mobile phases) reported in the literature, either the number of compounds that can be measured in limited, the run is prolonged if all metabolites are measured or the sensitivity is low without sample clean-up.

We have now developed a solvent system consisting of 0.1 M citric acid, 0.06% diethylamine, 0.05 mM $Na_2EDTA$, 200 mg/l 1-heptansulfonic acid and 3% acetonitrile at pH 2.5. With this solvent system and a 25 cm, 5µM ODS column, we can separate and analyze 12-14 biogenic amines and their neutral and acid metabolites during a single run lasting less than twenty minutes. The amines and metabolites include: norepinephrine (NE), 4-hydroxy-3-methoxyphenylglycol (MHPG), normetanephrine (NM) and metanephrine (MN); dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), 4-hydroxy-3-methoxyphenyl-acetic acid (HVA) and 3-methoxytyramine (3-MT); serotonin (5-HT), 5-hydroxyindole-3-acetic acid (5-HIAA) and 5-hydroxytryptophol (5-HTL). L-3, 4-dihydroxyphenylalanine (DOPA), 5-hydroxy-L-tryptophan (5-HTP) and epinephrine (EPI) can also be measured. We have used isoproterenol (IP) and 5-hydroxyindole (5-HI) as two internal standards for catechols and indoles respectively and both these are separated from the endogenous compounds. The brain tissue from the rat or mouse is homogenized with 5% perchloric acid (PCA) containing the desired internal standards (20 ng/ml). The homogenate is centrifuged at 5000 r.p.m. for 5 min and 50 µl of the clear supernatant is injected into the HPLC system.

One of the major advantages of the present system is the ability to quantitate MHPG which is distinctly separated from the solvent front. This is a problem in many solvent systems since MHPG elutes close to the solvent front and tends to be obscured by it. Further, this system provides quantitation of all metabolites of interest in biogenic amine metabolism thus providing increased amounts of data from each brain region analyzed. The sensitivity is in the picogram range thus making it possible to measure minor metabolites in any given tissue sample weighing 1-10 mg.

263.9 DIFFERENTIAL ALTERATIONS IN DOPAMINERGIC MARKERS IN THE BASAL FOREBRAIN AND STRIATUM OF RATS WITH 6-OHDA-LESIONS OF THE SUBSTANTIA NIGRA. C.Geula* and J.T. Slevin.Veterans Administration Medical Center and the Depts. of Neurology and Pharmacology and the Sanders-Brown Research Center on Aging, University of Kentucky, Lexington, Kentucky 40536

The aggregate from several laboratories suggests the presence of dopamine (DA), HVA and DOPAC (metabolic derivatives of DA), and DA receptors in the basal forebrain (substantia inominata, SI) of rats and primates including humans (nucleus basalis of Meynert, nbM). The present study investigates the source of the DA synaptic markers found in this region.

Sprague-Dawley rats were anesthetized and 8 ug of 6-hydroxydopamine was unilaterally injected in the substantia nigra pars compacta (SNc). After a week, the animals were sacrificed and striatal levels of dopamine and its metabolites were measured by high pressure liquid chromatography with electrochemical detection. The striatal dopamine level on the lesioned side was significantly decreased (-78%) compared with the nonlesioned side. The dopamine level was also significantly but more modestly decreased in the SI ipsilateral to the SNc lesion (-48%). DOPAC was significantly lower in the striatum and not detected in the SI (detection limits, 20pg) of the lesioned side. As documented from several laboratories, striatal $D_2$ receptor density on the lesioned side increased to 123% of control. In contrast, there was no change in ipsilateral $D_2$ receptor density in the SI of lesioned rats (-8%).

These data demonstrate that a lesion in the SNc leads to a decrease in DA levels in the ipsilateral SI without a concurrent denervation supersensitivity, in contrast to the well-documented receptor changes of the striatum which have been reproduced here. These findings suggest that the decrease in DA levels observed in the nbM of patient's with Alzheimer's disease may be due to partial destruction of DA nbM afferent projections from the SNc.

Supported in part by the VA Research Service, NINCDS (TIDA 00732) and NIA (1 T32 AG0084-01A1).

263.10 EFFECTS OF PCPA ON INDOLE CONCENTRATIONS IN BRAINSTEM AND SPINAL CORD TERMINALS. J.L. Steinman, S.M. Carlton, G. Hillman*, B. Haber and W.D. Willis. Marine Biomed. Inst. and Depts. Pharmacology and Physiology & Biophysics, Univ. Texas Medical Branch, Galveston, TX 77550.

Administration of para-chlorophenylalanine (PCPA) to inactivate the 5-HT rate limiting enzyme tryptophan hydroxylase produces profound reductions in indole concentrations in spinal cord samples. However, the depletion of 5-HT was less pronounced in nu. raphe magnus (NRM) and ventral cord as compared to nu. raphe pallidus (NRP) and dorsal cord, respectively. The present studies assessed whether regional changes in 5-HT after PCPA can be attributed to selective reduction in terminal structures.

5-HT in terminals was assessed with two approaches. First, 40 µm sections of brainstem (BS) and spinal cord (SC) from PCPA treated (600 mg/kg, IP for 3 days) or control rats were immunohistochemically (IHC) stained for 5-HT. Photographs were taken of NRM (directly dorsal to NRP), lamina II (dorsal) or lamina VIII (ventral) of lumbar cord through a microscope (40X). Negatives were viewed through a camera attached to a Grinnell image analysis system interfaced to a Masscomp MC500 computer. Images were digitized and analyzed with a program that identified only terminal structures. Pixels comprising nerve terminals were identified on the basis of their brightness as compared to neighboring pixels; axons, cell bodies and motoneurons were eliminated. The number of pixels (#-pix) falling within a brightness window was counted for each image and data grouped according to drug treatment. The #-pix representing IHC-stained terminals in NRM was reduced from control values by 46% after PCPA. The #-pix in lamina II was reduced by 69% and in lamina VIII by 49%. The overall analysis suggests that PCPA produced a greater reduction of 5-HT immunoreactive terminals in dorsal cord as compared to terminals impinging upon cells in NRM or on ventral horn motoneurons.

The second approach taken was to measure the concentration of 5-HT in synaptosomal fractions from BS and SC. Rats were pretreated with 400 mg/kg PCPA and sacrificed by decapitation. Tissue pooled from 2-4 rats/group was subjected to subcellular fractionation according to Whitaker et al. (1964). Aliquots of each fraction were taken for analysis of indoles and catecholamines using HPLC with a 3µ C18 column and coulometric (ESA) electrochemical detection. The 5-HT content of the crude P2 fraction was taken to represent synaptosomal 5-HT. PCPA differentially reduced 5-HT content of the BS fraction by 75% and SC fraction by 83% with parallel changes in 5-HIAA. These data corroborate the differential effects of PCPA on IHC stained terminals in NRM and SC. Conflicting effects of PCPA in behavioral or electrophysiological paradigms may be due to alterations at particular terminal sites.

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263.11 SPINAL CORD SUBSTANCE P RECEPTORS ARE PRESENT ON AUTONOMIC AND SOMATIC MOTOR NEURONS: STUDIES WITH SUICIDE TRANSPORT, MEDULLARY LESIONS AND NEUROTOXINS. C.J. Helke*, C.G. Charlton and R.G. Wiley. Uniformed Services Univ. of the Health Sciences, Bethesda MD 20814 and Vanderbilt Univ., Nashville, TN 37202.

Substance P (SP) receptors are present in spinal cord nuclei that contain motor neurons innervating somatic muscles or autonomic ganglia (J. Neurosci., in press). Our previous study (Brain Res. 328:190, 1985), using suicide transport of ricin combined with receptor autoradiography of $^{125}$I-Bolton Hunter-labeled SP, showed that in certain medullary nuclei SP receptors are present on motor neurons. In the current study, the cellular location of the SP binding sites in the intermediolateral cell column (IML), the phrenic motor nucleus, and the lateral part of the lumbar ventral horn (site of sciatic motor neurons) was investigated.

Two weeks after a unilateral injection of ricin into the superior cervical ganglion (SCG) of adult rats, SP binding and cholinesterase-stained neurons were reduced in the IML. Unilateral electrolytic lesions of ventral medullary SP neurons (which decrease SP content in the IML) do not alter the density of SP binding in the IML. Likewise, 6-hydroxydopamine and 5,7-dihydroxytryptamine which reduce the norepinephrine and serotonin contents of the spinal cord, do not reduce the SP binding in the IML. Whereas ricin injected into the SCG destroyed the preganglionic motor neurons and also altered the SP binding, the reduction of SP, norepinephrine and serotonin innervation to the IML failed to reduce binding. This suggests that the SP receptors are located postsynaptically on preganglionic neurons rather than presynaptically on SP (autoreceptors), or monoamine neurons.

Unilateral injections of ricin into the phrenic nerve resulted in the unilateral destruction of phrenic motor neurons in the cervical spinal cord and caused a marked reduction in the SP binding in the nucleus. Likewise, sciatic nerve injections of ricin caused a loss of associated motor neurons in the lateral portion of the ventral horn of the lumbar spinal cord and a reduction in the SP binding. Ricin-induced destruction of neurons is not always associated with a loss of SP binding sites. Sciatic nerve injections also destroyed afferent nerves of the associated dorsal root ganglia and increased the density of SP binding in the dorsal horn.

These data show that SP receptors are present on somatic and autonomic motor neurons of the spinal cord. Other tachykinin peptides such as substance K, neuromedin K, and eledoisin are not discretely localized in areas which contain spinal cord autonomic and somatic motor neurons (Buck et al., this meeting). (Supported by NIH Grant #NS-19317 to C.H. and VA Merit Review Grant to R.W.)

263.12 SUBSTANCE P RECEPTORS SHOW DEVELOPMENTAL CHANGES IN THE RAT: AN ONTOGENY STUDY. C.G. Charlton* and C.J. Helke, Uniformed Services Univ. of the Health Sciences, Bethesda, Maryland 20814.

Receptors for the polypeptide, substance P (SP), have been mapped and characterized in the spinal cord of rats. High density receptors occurred in sensory, autonomic and somatic motor nuclei in adult rats. Functional studies show a role for SP in sensory, autonomic and motor controls. These functions show postnatal developmental changes, which if mediated by SP, suggest that the receptors for the peptide may also undergo postnatal changes. This hypothesis was tested by using light microscopic autoradiography and membrane homogenate binding of $^{125}$I-Bolton-Hunter SP ($^{125}$I-BH-SP) to study SP receptors in the spinal cord of rats of different ages.

In cervico-thoracic segments of rat spinal cord, the autoradiograms showed that specific binding of $^{125}$I-BH-SP occurred exclusively in the grey matter and that mean binding varies inversely to age. In pups, up to about 15 days old, binding sites were generally diffusely distributed over the grey matter, and become progressively more defined in specific nuclei as the rats matured. The age-related binding was confirmed in membrane homogenate binding study of whole spinal cord, which showed that the ratio for the concentration (cpm/mg protein) of specific binding was 88: 12: 3: 1 for rats 11 (26gm), 38 (145gm), 90 (329gm) and 260 (553gm) days old. The ratio for the specific binding to the spinal cord (not corrected for tissue weight) for the same group of rats, was 22: 8: 3: 1. These data showed that SP receptors decreased as a function of age, and that the decrease in concentration was not entirely due to increases in protein.

These findings may represent a new avenue in the study of geratology, and developmental biology and in diseases e.g. sudden infant death syndrome (SIDS). Interestingly, the phrenic (respiratory) motor nucleus receives a bulbospinal SP projection and contains SP receptors. Recently, it has been shown that SP is elevated in the medulla of SIDS victims (Bergstrom et al., Brain Res. 323: 279-285, 1985). (Supported by NIH grant # NS-19317).

263.13 AUTORADIOGRAPHIC LOCALIZATION OF BOMBESIN BINDING SITES WITHIN THE GASTROINTESTINAL TRACT. Timothy H. Moran, Terry W. Moody, Michael S. Goldrich*, Paul H. Robinson and Paul R. McHugh. Department of Psychiatry, The Johns Hopkins University School of Medicine, Baltimore, MD 21205, Department of Biochemistry, The George Washington School of Medicine and Health Sciences, Washington, D.C. 20037.

Bombesin (BN) is a tetradecapeptide with biological activity in both the gastrointestinal (GI) tract and brain. Peripherally administered bombesin has been demonstrated to inhibit food intake and this action of BN appears to be peripherally mediated. In order to identify candidate target sites at which BN could exert this effect, the distribution of BN binding sites was mapped in the stomach and small intestine by *in vitro* autoradiography.

Binding sites for BN were labelled with $^{125}$I Tyr$^{4}$ BN. Binding was carried out on 25u slide mounted tissue sections taken from the fundus, antrum, pylorus, duodenum and ileum. Binding conditions were similar to those demonstrated to provide high affinity binding to brain sections with specific binding of 80-85%. Tissue sections were incubated for 60 min at 22°C in 10 mM HEPES, pH 7.4, 130 mM NaCl, 4.7 mM KCl, 5 mM MgCl2, 1mM EGTA, 0.5% bovine serum albumin, 0.07 mM bacitracin and 3nM $^{125}$I BN in the presence or absence of 1 uM unlabeled BN. Incubations were followed by two consecutive 4-min washes in buffer at 4°C. Slides were dried and apposed to LKB H$^{3}$ Ultrofilm to obtain autoradiographic images.

The greatest density of binding was found in the gastric fundus. Binding was also present in the proximal gastric antrum and in the duodenum and ileum. In all cases binding appears to be limited to the neural plexus within these tissues and is not found in either the mucosal or muscular layers. No binding was found within the pyloric sphincter. This pattern of binding sites is clearly differentiated from that of cholecystokinin (CCK), another peptide which inhibits food intake. CCK receptors are exclusively localized to the circular muscle layer of the pyloric sphincter in adult rats. The differential distribution of BN and CCK binding sites within the GI tract suggests that CCK and BN exert their effects on food intake through different sites of action.

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264.1 MULTIPLE, LONG-RANGE FOCAL COLLATERALIZATION OF SINGLE CORTICO-CORTICAL AXONS IN MONKEY SENSORY-MOTOR CORTEX. J. DeFelipe*, M. Conley and E. G. Jones. Department of Anatomy, University of California, Irvine, CA 92717.

Vertical (interlaminar) connectivity dominates thinking on the intrinsic organization of the cerebral cortex. Horizontal (intralaminar) connectivity also exists and axoplasmic transport studies reveal inter- and intra-areal connections terminating in multiple dissociated clusters of axonal arborizations which arise from similarly dissociated patches of pyramidal neurons. We sought to determine whether individual cortico-cortical axons terminate in multiple focussed interareal and intraareal arborizations corresponding to the patches.

We made small iontophoretic or pressure injections of horseradish peroxidase (HRP) in the white matter just beneath area 3b or 5. We then reconstructed retrogradely labeled pyramidal cells in which axons and collaterals could be traced from somata for distances of up to 6 mm.

The cell bodies of the cells were located in layer III of areas 4, 3a, 3b, 1, 2, or 5. The axon descended vertically towards the white matter and during the initial trajectory the axon measured 1-1.5 μm but at a distance of 20-30 μm from the cell body where the axon become myelinated it measured 2-5 μm. Collaterals of the main descending axons were of two kinds: one was very thin and within a few microns devolved into terminal branches with small boutons terminaux; the others were major, myelinated collaterals which had long horizontal or oblique trajectories traversing several cytoarchitectonic areas. There were two distinctive characteristics of the major collaterals: first the long trajectory (sometimes over 800 μm) over which they gave off no branches; second, several terminal branches arising from widely separated parts of the parent collateral and even from different major collaterals of the same cell could converge on the same cortical focus, giving rise to dense patches of terminations mainly in layers II-IV. Single cells were observed that had one to three terminal patches in their own cytoarchitectonic area as well as one or more patches in one to four of the other areas, the connectivity between areas following previously established rules (Jones et al. J. Comp. Neurol. 181: 291, 1978).

The present results indicate a highly specific spread of connections in the horizontal dimension of the primate sensory-motor cortex formed by single axons and which contrasts with the pattern of equally specific vertical connections formed by certain populations of pyramidal and non pyramidal cells. (Supported by Grants Numbers NS 21377, NS 507072 and F05 two 3156 from the NIH, USPHS).

264.2 EXPANSION OF THE S-I CORTICAL AREA ACTIVATED BY SAPHENOUS NERVE INPUTS IN RATS AFTER DESTRUCTION OF SCIATIC NEURONS WITH THE LECTIN RICIN. J. T. Wall, R. G. Wiley, and C. G. Cusick. Depts. of Psychology and Neurology (VAMC), Vanderbilt University, Nashville, TN 37240.

When the hindpaw skin of an adult rat is partially deafferented by mid-thigh sciatic nerve section, regions of S-I cortex usually representing sciatic inputs become responsive to hindpaw zones which remain innervated by the saphenous nerve (J. Neurosci., 4, 1984, 1499). Since the cell bodies and central processes of primary sensory neurons commonly survive distal nerve section, it is possible this enlargement in the saphenous projection system is actively modulated by some signal from sectioned sciatic neurons. In the present study, we attempted to eliminate sciatic neurons to evaluate what effect removal of primary sensory neurons and possible active signal release has on the size of the cortical projection of saphenous inputs.

Dorsal root ganglion neurons of adult rats were destroyed as a result of retrograde transport of the toxic lectin ricin from mid-thigh injections of the sciatic nerve. Previous anatomical studies indicate such injections produce a severe loss of cell bodies in dorsal root ganglia associated with the sciatic nerve (Soc. Neurosci. Abstr. 9, 1983, 298). After ricin injection, maps of the S-I hindpaw representation were made from neurophysiological recordings and compared to hindpaw maps from normal and sciatic-sectioned rats.

The results indicate the cortical projection area of saphenous inputs in rats that had ricin injections 6-101 days before mapping ($\bar{x}$ = .57 mm$^2$) is: larger than the saphenous area of normal rats ($\bar{x}$ = .14 mm$^2$; $t(15) = 10.44$; $p < .001$), and not statistically different from the saphenous area of rats whose sciatic nerves were sectioned and ligated 4-91 days before mapping ($\bar{x}$ = .45 mm$^2$; $t(11) = 2.02$; $p > .05$). In addition, plots of the size of the saphenous area as a function of time after ricin injection or section are similar. Thus, the spatial and temporal features of adjustment in the size of the cortical projection of saphenous inputs appear comparable after section of sciatic processes and after loss of sciatic neurons.

Although it is recognized that injury of one part of the nervous system commonly leads to organizational adjustments in related circuits which survive damage, there is little understanding of the factors controlling such adjustments. The present findings suggest primary sciatic neurons do not actively modulate size adjustments in the cortical projection system of neighboring saphenous inputs during the first 100 days after sciatic injury.

264.3 MORPHOLOGY AND ULTRASTRUCTURE OF TERMINATIONS FROM SINGLE CORTICOSPINAL TRACT AXONS ARISING FROM SENSORIMOTOR CORTEX IN THE CAT. E.J. Casale*, A.M. Kavookjian*, A.R. Light (SPON: D. McIlwain). Department of Physiology, UNC-Chapel Hill, Chapel Hill, NC 27514

Little is known concerning the laminar collateral distribution and synaptic ultrastructure of single, corticospinal tract (CST) axons. In the present experiments, single CST axons arising from sensory cortex were impaled with micropipettes filled with 4-10% horseradish peroxidase (HRP), near their termination within the lumbar enlargement in chloralose-anesthetized cats. Axons were positively identified as corticospinal by their ability to "follow" electrical stimulation of the cortex at 300 Hz with minimal (<0.1ms) latency shifts.

Conduction velocities ranged from 9-112 m/sec with a mean of 37 m/sec. Typically, individual CST axons activated via natural stimulation responded to brush or tap of the entire leg, foot, and tail contralateral to its origin in the cortex. Occasionally units had spontaneous activity. Intraaxonally stained fibers displayed wide variation with respect to the amount of collateralization within the gray matter, their mediolateral and rostrocaudal extent, and their laminar distribution. Overall, however, boutons were found mainly in laminae III through VII.

Boutons of one fiber were analyzed at the EM level. Both boutons en passant and boutons terminaux were found within laminae III, IV, and V. Most boutons were large and glomerular-shaped with preterminal axons myelinated as far as the glomerulus. Asymmetrical contacts were made with many dendritic spines. Occasionally contacts were made on large dendritic shafts, but no axo-axonic or axo-somatic contacts were found. Vesicles were mostly round (40nm diameter) and clear, except for the occasional presence of a few large dense-core vesicles (85nm diameter).

These data indicate that CST axons, as well as primary afferent axons, may terminate within the dorsal horn as glomeruli.

This research was supported by NS-20339.

264.4 SYNAPTIC CONNECTIVITY OF THALAMOCORTICAL AXON TERMINALS WITH SPINY STELLATE NEURONS IN MOUSE SmI CORTEX. G. Benshalom* and E. L. White. (SPON: N. Vardi). Unit of Morphology, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva 84105, Israel.

The distribution of thalamocortical (TC) synaptic connections with spiny stellate cells in layer IV of the barrel region in mouse primary somatosensory cortex, was examined in 7 three month-old, male CD/1 mice. TC afferents were labeled by lesion induced degeneration, which, in previous studies, had been shown to label reliably all TC synapses in mouse barrel cortex. Golgi impregnated/gold-toned spiny stellate cells were identified with the light microscope prior to thin sectioning. Analyses of 7 spiny stellate cell dendrites from 7 different neurons showed them to form most of their synapses with their dendritic spines rather than with their shafts. The relatively few synapses on dendritic shafts were about evenly divided between symmetrical and asymmetrical types; spinous synapses were primarily of the asymmetrical type. From 13 to 23% of all asymmetrical synapses with spiny stellate cell dendrites were with TC axon terminals and these were located mostly on spine heads. Spines forming TC synapses were irregularly distributed along the entire lengths of the dendrites, except for their proximal segments which bore few spines. These results show that spiny stellate cell dendrites form a relatively high proportion of TC synapses as compared with other types of neurons in mouse SmI cortex, implying that spiny stellate cells are strongly influenced by TC synaptic input. Moreover, the efficacy of spinous synapses may be enhanced by active membrane properties of the spine head. Thus the possibility exists that by virtue of their spines, thalamic input may influence layer IV spiny stellate cells to an even greater extent than would be predicted merely by the distribution of TC synapses.

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264.5 PROJECTIONS OF THE MEDIAL PORTION OF THE POSTERIOR THALAMIC NUCLEAR GROUP (POM) TO THE SOMATOSENSORY CORTEX OF THE RAT. K.A.Koralek*, K.F.Jensen and H.P.Killackey*. (SPON: L.Ide) Dept. of Psychobio., Univ. of Calif., Irvine, CA 92717.

In the somatosensory cortex of the rat two cytoarchitectonic zones can be distinguished. The granular zone has a well developed layer IV and receives clustered projections from the ventral posterior nucleus (VP). The dysgranular zone has a poorly developed layer IV and has been described as receiving sparse thalamic input. The present study focuses on the projections of POM to somatosensory cortex.

Adult Sprague Dawley rats were injected with .1 to .5 microliters of WGA-HRP into the dysgranular zones. The animals were perfused after a 12 to 24 hour survival period. Coronal sections were processed with TMB. Additional rats were injected with .25 to .75 microliters of WGA-HRP into either POM or VP. The cerebral hemispheres were flattened, sectioned tangentially and processed with TMB.

Injections into the dysgranular zone result in extensive retrograde labeling in POM. The bulk of labelled cells are located in the anterolateral aspect of POM bordering on the dorsal and medial borders of VP. Injections of WGA-HRP into POM produces different patterns of label in the dysgranular and granular zones. The anterograde label was heaviest in the dysgranular zone. In some cases, a uniform dense label filled a substantial portion of the dysgranular zone adjacent to the barrel field. This area was devoid of anterograde label following large injections of WGA-HRP into VP. In the adjacent granular zone, injections of POM resulted in sparse label distributed in a grid-like pattern complementing the pattern of anterograde label from VP. This distribution of label is open to two interpretations. It may reflect either direct afferents from POM or collaterals arising from corticothalamic projection neurons projecting to POM from somatosensory cortex.

These results indicate that POM is a major source of thalamic input to the dysgranular zone. We interpret this finding as evidence that POM and VP project differentially to the rodent somatosensory cortex.

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264.6 AT WHAT LEVELS OF THE SOMATOSENSORY SYSTEM DOES MI CORTEX STIMULATION MODULATE CUTANEOUS SENSORY TRANSMISSION IN THE RAT? S.E. Knowles, H.-C. Shin and J.K. Chapin. U. of Texas Health Sci. Cntr., Dallas Tx. 75235.

We have used standardized electrical stimulation parameters in the forelimb area of MI cortex to test motor cortical modulation of cutaneous sensory responses in the following somatosensory relays: cuneate nucleus, VPL thalamus, and SI cortex (granular and infragranular layers.) In 36 ketamine anesthetized rats (200-250 gm) a narrow (.1 mm diam) bipolar concentric stimulating electrode was driven to a depth of 1700 uM into a standard location in the MI cortical forelimb area (2.0 mm anterior to bregma, 3.5 mm lateral to midline). The same stimulation parameters (single 1.0 msec pulses; 50 uAmp constant current) were used for all experiments. These currents were lower than thresholds for producing forelimb movement (average about 100 uA). This MI stimulation was used as the conditioning (C) stimulus in a condition-test (C-T) paradigm in which the test (T) stimulus was standardized mechanical vibration of digit #2 of the contralateral forepaw. Post-stimulus histograms were used to quantitate the responses to these C and T stimuli delivered at different C-T intervals.

In the cuneate nucleus, direct responses to the MI cortical stimulation were rare ( <10% of animals). However, in about 50% of recorded cells the C-stimulation (in MI cortex) caused a moderate inhibition of responses to T-stimulation (on forepaw). This inhibition mainly occurred at longer C-T intervals ( >25 msec). Most cells in the cutaneous forepaw area of the VPL thalamus were similar to those in the cuneate in that they did not respond directly to the MI cortical stimulation, but their responses to paw vibration were moderately inhibited by C-stimulation at relatively long C-T intervals. A few cells, however, especially those in VPL locations representing more proximal limb, or head areas, did respond to the MI stimulation. These exhibited a more powerful, and shorter latency C-stimulus induced inhibition of responses to T-stimulation (of appropriate skin areas). Similar C-T interaction studies were carried out during microelectrode penetrations through the layers of the SI cortical forepaw area (2-3 mm away from the stimulating electrode in MI cortex). Very few neurons in the granular or supragranular layers responded directly to the MI stimulation, but in the infragranular layers both ortho- and antidromic responses were common. These infragranular cells were much more strongly inhibited by C-stimulation at short (10-25 msec) C-T intervals. In conclusion, the MI cortex appears to exert stronger effects on somatosensory transmission within the SI cortex than at lower relays. Supported by grants NS18041, AA0390, and the Biological Humanics Foundation.

264.7 MORPHO-FUNCTIONAL CORRELATION ON N. RETICULARIS THALAMI NEURONS:AN INTRACELLULAR AND HRP STUDY IN RAT'S THALAMIC SLICES. R. Spreafico*, M. De Curtis*,C. Frassoni*,G. Avanzini*(SPON: European Neuroscience Association) Dept. of Neurophysiology - Ist. Neurologico "C. Besta" - 20133 Milano (Italy).

Aim of the present work is to investigate the intrinsic organization of n. Reticularis thalami (Re) by means of a combined anatomical and physiological study using intracellular recording and HRP injection of single neurons. 20 experiments were performed on horizontal slices (300-400 μm thick) of rat thalamus incubated in vitro. Glass micropipettes filled with $K^+$ acetate (4M) or with a 4% Horseradish Peroxidase (HRP) solution in 0.2 M KCl were employed for intracellular recording and intracellular injection of the enzyme. Resistances of microelectrodes were 40-80 MΩ and 80-150 MΩ respectively. Recording electrodes were positioned in the n. Re. Recorded cells were identified physiologically by orthodromic stimulation of internal capsule and ortho- or antidromic activation from the thalamus using tungsten stimulating electrodes. After the physiological study, HRP was intracellularly injected through the recording micropipette by means of positive pulses (2-3 nA, 10 msec in duration, 50 Hz for 2-3 minutes). Two hours after injections, slices were immersed in phosphate buffer solution containing mixed aldehydes for 2 hours and then transferred in a 30% sucrose phosphate buffered solution. After fixation slices were cut in 100 μm thick frozen sections and processed for HRP visualization. Camera lucida drawings were performed on serial sections in order to reconstruct HRP injected neurons.

25 out of 40 impaled neurons were orthodromically activated by both I.C. and thalamic stimulation and showed similar membrane characteristics. Twelve neurons were also labelled by HRP and camera lucida reconstructed. Pericarya of these cells were generally fusiform with dendrites arborizing mainly in the anteroposterior extent of the nucleus. Few spines were visible. The axons of labelled neurons were reconstructed for long distance in different thalamic nuclei. In few instances axon collaterals emerging from the proximal portion were observed to ramifie within the Re.

The results suggest the existence of a subpopulation of Re projecting neurons subserving also intrinsic local circuitry.

264.8 INTRACORTICAL AND THALAMIC CONNECTIONS OF SMI FOREPAW CORTEX OF THE RACCOON. G.S. Doetsch, K.W. Johnston*, G.P. Standage and C.-S. Lin. Depts. of Surg. (Neurosurg.), Physiol. and Anat., Med. Coll. Ga., Augusta, GA 30912 and Dept. of Anat. Duke Univ., Durham, NC 27710.

Our previous electrophysiological studies have shown that denervation of a forepaw digit in the adult raccoon causes neurons within the deprived portion of SmI cortex to develop "new" receptive fields (RFs) on digits adjacent to the denervation site. Because some of these changes occur within 24 hours, we suggest that they reflect the unmasking of pre-existing anatomical connections.

To test this notion, the intracortical and thalamic connectivity of SmI cortical digit zones was examined in adult raccoons using the horseradish peroxidase (HRP) tracer method. Injections of 50% HRP (.01-.3 ul) were made into the crown of a specific gyrus representing digit 2, 3, 4 or 5. After 48 hours, each animal was perfused intracardially with saline followed by an aldehyde mixture, and the brain was processed for HRP histochemistry. The locations of retrogradely-labeled neurons and orthogradely-labeled axon terminals in the thalamus and SmI cortex were plotted.

The gyral crown of each SmI cortical digit zone was found to have specific reciprocal thalamic connections with a distinct crescent-shaped lamella of neurons extending rostrocaudally through the lateral part of VPL. The regions containing labeled thalamocortical relay neurons and corticothalamic terminals overlapped extensively. Neurons within adjacent lamellae were not labeled, consistent with the finding by Rasmusson (1985) that, in the raccoon, VPL relay neurons project their axons to only one cortical digit area with no collaterals to neighboring areas.

In contrast, the SmI cortical digit zones themselves were found to be interconnected by local cortico-cortical circuits. The gyral crown of a given zone was reciprocally connected with small clusters of neurons located in the fundus of one or both sulci demarcating that gyrus and separating it from adjacent digit zones. The cells were confined mainly to layer VI, while the axon terminals were located primarily in layers III-IV and VI. Shorter reciprocal connections were found with other neuronal clusters located within the crown or sulcal banks of that same gyrus. These cells and axon terminals were present mainly in layers II-IV and VI. Thus, a given digit zone can receive input from neighboring digit areas via neurons located in the sulcal regions demarcating that zone.

The action of these cortico-cortical connections may largely account for the unmasking of "new" RFs following denervation. Projections from adjoining digit zones to the intervening sulcal region could account for the convergent functional properties of neurons located in that region. Input from the sulcal regions may then explain why the crown of a reactivated digit zone develops convergent properties similar to those of normal sulcal regions.

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264.9 QUANTIFICATION OF THALAMOCORTICAL AND CORTICOCORTICAL AXON TERMINALS USING PHA-L IMMUNOHISTOCHEMISTRY. E. L. White and A. Keller*. Unit of Morphology, Faculty of Health Sciences, Ben Gurion University, Beer Sheva, Israel

Phaseolus vulgaris leuco-agglutinin (PHA-L) was injected iontophoretically into the vibrissal area of either the ventrobasal thalamus or of the ipsilateral SII area in CD/1 mice. After perfusion, cortex ipsilateral to the injection site was sectioned and reacted with biotinylated anti-PHA, with an avidin-biotin-horseradish peroxidase complex and then with diaminobenzidine to demonstrate the horseradish peroxidase. The result of this procedure was to label thalamocortical (TC) and corticocortical axons and their terminals with a reaction product visible with both the light and electron microscopes. Results of the TC study confirmed previous findings that TC axon terminals form about 20% of the asymmetrical synapses in layer IV of mouse barrel cortex. This suggests that the anterograde PHA-L method can provide accurate information on the numbers and types of synapses involving identified axon terminals. In addition, because preterminal axon segments remain intact and are labeled, it should be possible to use the anterograde PHA-L method to determine how many synapses are made by a single axon, and further, how many axons contact individual postsynaptic elements. However, it cannot be excluded that some PHA-L labeled terminals are collaterals of cells labeled by the retrograde transport of the lectin. Therefore, as a further check on the efficacy of PHA-L as an anterograde label, we compared results using PHA-L with results of combining HRP injections and lesions at a single site. The latter approach enables afferents, identified by lesion induced degeneration, to be differentiated from collaterals labeled by the retrograde transport of HRP. Results of lesion/injections at sites in thalamus and SII will be presented. Supported by N.I.H. # NS 20149-02, BSF # 3201/83.

264.10 SPATIOTEMPORAL RESPONSE PROPERTIES OF SOMATOSENSORY NEURONS IN THE PERIPHERY AND SI CORTEX OF THE MONKEY MEASURED WITH SCANNED, VARIABLY SPACED DOT ARRAYS. J.R. Phillips*, K.O. Johnson, K.H. Fasman, S.S. Hsiao*. (SPON: A.B. Schwartz) Bard Laboratories, Dept. Neuroscience and Dept. Biomedical Eng., The Johns Hopkins University Sch. Medicine, Baltimore, Md. 21205.

The spatial and temporal resolution of peripheral and cortical neurons was investigated using plastic surfaces composed of arrays of raised dots with center-to-center spacing varying from 0.9 to 6.2 mm and dot diameters ranging from 0.4 to 1.2 mm. The dot heights were 0.6 mm. In the peripheral experiments, monkeys (M. mulatta) were anesthetized with sodium pentobarbital and action potentials were recorded from single afferent axons. In the cortical experiments the monkeys were alert and performing a behavioral task that was unrelated to the tactual stimuli. Their hands were restrained in an apparatus that allowed stimuli to be applied to the distal pads of the fingers. The dot patterns were swept across the finger pads repeatedly in such a manner that the receptive field scanned the entire surface in successive sweeps. When mapped onto the coordinates of the stimulus surface, the action potential patterns provide a direct display of the spatiotemporal resolution of the neuron.

Neurons studied in this way showed greater resolution than has been reported previously for similar patterns. The minimum center-to-center dot spacing resolved by peripheral slowly adapting afferent fibers ranged from 1.1 mm for the most sensitive fiber to 2.4 mm for the least sensitive fiber. The comparable range for quickly adapting cutaneous afferents was 1.8 to 3.4 mm. Pacinian afferents had responses that could not be easily classified; individual dots at even the largest spacings were not resolved, but these responses were not entirely unstructured. Scanning velocity (20-100 mm/sec) had little, if any, effect on the resolution of peripheral afferents. Likewise, dot diameter had relatively little effect on the maximum resolution of the peripheral afferents. Cortical neurons studied in SI cortex, predominantly in area 1, varied greatly in their capacity to resolve these stimulus patterns with minimum resolved dot spacing ranging from 1.1 to 6.0 mm. The most sensitive cortical neuron (a neuron with a sustained response to steady cutaneous deformation) was as sensitive to spatial detail as the most sensitive peripheral afferents. The small population of cortical neurons studied using a range of scanning velocities showed a mild loss of spatial resolution with increased scanning velocity. Supported by NIH Grants NS18787 and GM07057.

264.11 NEURAL REPRESENTATION OF EMBOSSED LETTERS IN PERIPHERAL MECHANORECEPTIVE AFFERENTS AND SOMATOSENSORY CORTEX OF THE AWAKE MONKEY. S.S. Hsiao, K.H. Fasman, J.R. Phillips*, and K.O. Johnson. Bard Laboratories, Dept. Neuroscience and Dept. Biomedical Eng., The Johns Hopkins University Sch. Medicine, Baltimore, MD. 21205.

The human capacity for tactual spatial pattern recognition has previously been investigated in psychophysical experiments using embossed letters of the alphabet with heights ranging from 3.0 to 8.0 mm. The experiments reported here employ the same stimuli and are aimed at studying the neural representation of letters and the transformations effected in the ascending somatosensory pathways. The experiments fall into two categories. In the peripheral experiments, recordings were made from single afferent axons of monkeys (M. mulatta) anesthetized with sodium pentobarbital. In the cortical experiments, recordings were made from neurons located in SI cortex of alert monkeys performing a behavioral task unrelated to the test stimuli.

The stimuli consisted of 3 full alphabets (2.0, 4.5, and 6.5 mm high) and a set of 10 A's and S's ranging in height from 2.0 to 10.8 mm. The letters were swept repeatedly across the finger pads, in such a manner that the receptive field scanned the entire surface in successive sweeps. When mapped onto the coordinates of the stimulus surface, the action potential patterns provide a direct display of the neurons' spatiotemporal responses to the embossed patterns. The peripheral slowly adapting (SA) afferents resolved 6.5 and 4.5 mm alphabets quite effectively, but at 2.0 mm the representation was markedly degraded. Quickly adapting (QA) afferents required patterns about 50% larger for their spatial resolution to equal that of the SA afferents. However, a large number of the 4.5 mm letters could be recognized from QA spatiotemporal responses, suggesting that these afferents may play some role in tactile spatial pattern recognition. Changes in scanning velocity (from 20 to 40 mm/sec) had no effect on the spatial response patterns of the SA's and had a minor effect on the response patterns of some QA afferents. The Pacinian afferents resolved the patterns poorly and we infer that they play no role in tactile spatial pattern recognition.

Neurons studied in SI cortex, predominantly area 1, yield spatiotemporal responses that are different from those seen in the periphery. In most cases it is not possible to identify the stimulus by simple visual inspection of the neuronal responses. This is so even for cortical neurons that resolve periodic spatial patterns as effectively as the most sensitive peripheral SA afferents. Supported by NIH Grants NS18787 and GM07057.

264.12 ROUGHNESS PERCEPTION: A PSYCHOPHYSICAL AND NEUROPHYSIOLOGICAL INVESTIGATION. K.H. Fasman, S. Hsiao*, J.R. Phillips*, and K.O. Johnson. Bard Laboratories, Dept. Neuroscience and Dept. Biomedical Eng., The Johns Hopkins University Sch. Medicine, Baltimore, MD. 21205.

The neural mechanisms underlying tactile roughness perception were investigated using 18 plastic surfaces composed of arrays of raised dots. Three dot diameters (0.4, 0.8, and 1.2 mm) and six center-to-center spacings (1.3, 2.4, 3.2, 4.3, 5.2, 6.2 mm) were used. In the psychophysical experiment subjects explored each surface with the pad of the index finger, using whatever force and velocity seemed most appropriate, and reported their subjective estimate of roughness. The surfaces were presented in random order and hidden from view. Normalized roughness estimate versus dot spacing reached a maximum at 3.2 mm and then fell again at larger dot spacings, i.e. the surfaces began to be perceived as smoother at greater dot separations. The effect of dot diameter was much more pronounced at small dot spacings than at larger spacings. At the smallest dot spacing, 1.3 mm, the medium and small dot diameters produced roughness estimates that were 2.5 and 6 times greater than the estimates for the largest dot diameter. At large dot spacings, 3.2 mm and above, dot diameter had little effect, although increasing dot diameter still reduced the subjective estimates slightly.

The same stimuli were repeatedly scanned across the receptive fields of macaque peripheral mechanoreceptive afferents (slowly adapting (SA), quickly adapting (QA) and Pacinian (PC)). The responses of the SA and QA afferents were spatially and temporally modulated for all dot spacings of 2.4 mm and greater. At the smallest dot spacing (1.3 mm) some of the the SA afferents were modulated effectively. The Pacinian afferents produced very complex responses which were clearly patterned but not at the fundamental spatial and temporal frequencies of the stimulus. It is clear that subjective reports of roughness were not related to any simple aspect of the spatial or temporal patterning in the afferent discharge. Surfaces producing equal reports of subjective roughness, e.g. 0.4 mm dots at 1.3 mm spacing and 0.8 mm dots at 6.2 mm spacing, produced very different spatial and temporal neural response patterns in all of the afferents. However, mean impulse rate in all of the afferent populations behaved in the same general manner as did subjective roughness, rising with increasing dot spacing to a maximum value and then falling. Supported by NIH Grants NS18787 and GM07057.

264.13 MECHANICALLY DRIVEN TRIGEMINAL EVOKED POTENTIALS IN CAT. Steven M. Barlow and Kevin Spangler*. Orofacial Physiology Laboratory, Boys Town National Institute, Omaha, NE 68131.

Afferent information derived from cutaneous mechanoreceptors distributed around joints and within areas of skin which undergo conformational changes associated with movement is thought to play an important role in providing feedback for developing and maintaining ongoing fine motor control. The purpose of the present study was to examine the response characteristics of the trigeminal system in cat to natural stimulation.

Short latency brain stem (far field) and cortical (early near-field) potentials were evoked by mechanical stimulation of the perioral face region and recorded epidurally over the primary sensorimotor cortex and directly from more caudal structures in the trigeminal lemniscal pathway, including the venteroposteromedial nucleus of the thalamus. Experiments were performed on adult cats positioned in a stereotaxic frame. They were anesthetized with nembutal and tracheostomized. Body temperature was monitored and maintained within resting levels. Monophasic displacements, with peak to peak rise times of approximately 5 milliseconds, were applied to the nonglabrous surfaces of the upper lip using a specially designed linear motor having a probe contact area of 0.125 $cm^2$. Stimulus amplitude ranged from 25 to 1000 microns. Multi-channel recording of far-field potentials were differential using Ag/AgCl ball macro-electrodes (0.5 mm tip diameter) for extradural recordings and platinum multiunit electrodes (50 micron tip diameter) for depth recorded potentials. Amplified signals and motor synchronization pulses were digitally signal averaged. The location of recording sites and extent of lesions were determined by histologic examination of stained serial sections through the damaged tissues.

Surface recorded cortical (early near-field) and brain stem (far-field) potentials were adequately evoked by the application of mechanical stimuli to the upper lip. Although more complex in waveform morphology, the mechanically driven trigeminal somatosensory evoked potentials (TSEP) obtained in the present study are similar to those recorded in response to direct electrical stimulation of the mental nerve (Dong, W.K., Brain Res., 233: 205-210, 1982). Additionally, the latencies and amplitudes of individual components of the mechanically driven TSEP varied systematically with the rate and amplitude of stimulation.

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264.14 SIMULATIONS OF A SPATIO-TEMPORAL MODEL FOR CORTICAL 2-DG PATTERNS. C. E. Smith* and D. G. Kelly* (SPON: B. L. Whitsel) Dept. of Statistics, Biomathematics Program, North Carolina State University, Raleigh, NC 27695 and Dept. of Mathematics, Univ. of North Carolina, Chapel Hill, NC 27514.

Recent labelling techniques, such as 2-deoxyglucose (2-DG), are beginning to provide insight into the spatial pattern of cortical activity. The amount of labelling incorporated is usually assumed to be directly related to the cummulative neuroelectric activity of the labelled neurons. Here we present simulated two dimensional patterns of firing rates (cortical activity) at a series of successive time intervals, e.g. 20, from a neural network model that emphasizes the modular nature of cortical processing (Szentagothai, J. Rev. Physiol., Biochem. Pharm., 98: 11-94, 1983). The goal of the modelling effort is to examine a variety of excitatory and inhibitory connection patterns between cortical columns and sub-columns and determine classes that produce steady-state patterns similar to those seen in 2-DG experiments. The specific application is to mammalian primary somatosensory cortex. To provide a greater degree of resolution than that of a cortical column (300 x 300 u), we use as our basic unit a cortical square of 150 x 150 microns. For each unit in a 2-D matrix, e.g. 52 x 52 units, excitatory and inhibitory connections to other units are assigned weights, reflect that unit's contribution to changes in the designated unit's average firing rate. For example, a connection pattern of a unit to its 48 nearest neighbors are specified. In addition to inputs from neighboring units, the 2-D matrix also receives a separate set of two dimensional inputs, called the input pattern and representing intraunit connections. The weighted inputs are summed and input to a sigmoidal function to incorporate firing rate saturation and zero clipping. For small signal analysis, the sigmoidal function is disregarded and methods of two dimensional signal processing are used.

For a fixed spatial input pattern, e.g. a 2-D ascending staircase, various connection patterns among units were examined. Center surround connections can produce edge enhancement, corner enhancement, peak detection, and damned temporal oscillations for different parameter values. Moving spatial input patterns were also examined as were altering connection weights due to level of activity (adaptive spatial filtering). The use of the model is intended to be a pragmatic one. First determine several connection pattern parameters that give the desired steady state solution patterns for a given spatial input pattern, then predict the response to different input patterns and pharmacological manipulation of connection parameters, e.g. remove a type of inhibitory synapse. (Supported by ONR Contracts: N00014-85-K-0105 and N00014-83-K-0387).

## OPIOIDS: PHYSIOLOGICAL STUDIES II

265.1 A NON-OPIOID MECHANISM AT THE LEVEL OF THE SENSORIMOTOR CORTEX MEDIATES THE ANTICONVULSANT EFFECT OF OPIATES IN THE RABBIT. M. Massotti and M.G. Spillantini* Laboratorio di Farmacologia, Istituto Superiore di Sanità, 00161, Roma, Italy.

Using various animal models of experimental seizures, data have been provided indicating an anticonvulsant effects of opiate agonists. In the rabbit, the convulsant effect due to the injection of penicillin (150 U.) into the sensorimotor cortex is prevented by administration of morphine (0.2-1 mg/kg iv) and cyclazocine (0.05-3 mg/kg iv). Naloxone, at doses (5-20 mg/kg iv) that per se slightly potentiate the convulsions due to penicillin, fails to antagonize the effect of morphine and cyclazocine (Massotti et al., Br. Res. 310: 201, 1984). On the contrary, it has been recently found that minute doses of naloxone (1-50 ug/kg iv) also display an anticonvulsant effect. The effects of the morphine and naloxone can be differentiated on the basis of the different EEG patterns of antagonism observed. While morphine inhibits the transmission of the ictal event, naloxone mainly inhibits the primary focus. Both are inhibited by cyclazocine, a mixed agonist-antagonist (manuscript in preparation).

On the basis of these results, it was worthwhile to study the effects of morphine, cyclazocine and naloxone injected directly into the sensorimotor cortex (ic). The three opiates were injected in a volume of 20 ul 5 min before penicillin (150 U. in 20 ul) in the same site. As shown in the table, the opiates reduce the incidence of the convulsions due to penicillin (150 U.).

| Drugs | pmoles ic x animal | C/T | Values x convulsant animal N. seizures | Total seizures time |
|---|---|---|---|---|
| Saline 20 ul | | 7/8 | 13.3 | 13.8 |
| Naloxone | 0.3 | 2/6 | 2.0 | 1.6 |
| Cyclazocine | 0.5 | 1/6 | 17.0 | 23.6 |
| Morphine | 60 | 4/9 | 6.1 | 2.5 |

C=N° animals showing convulsions T=N° animals treated

Moreover, in animals showing convulsions after naloxone and morphine, both the number of seizures and the total seizure time were also reduced. Unlike the effects observed after iv injection, no difference was observed among the three opiates in the EEG pattern of antagonism.

In conclusion, present data would suggest that in the sensorimotor cortex of the rabbit a non-opioid mechanism mediates the anticonvulsant effect of opiates.

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265.2 THE EFFECT OF IMMUNOSUPPRESSION AND OPIATES UPON THE VISUAL EVOKED RESPONSES OF CORTICAL AND SUBCORTICAL STRUCTURES. N. Dafny, P. Dougherty* and N.R. Pellis*. Department of Neurobiology and Anatomy, and the Department of Surgery, University of Texas Medical School, Houston, Texas 77030.

Previous studies from this laboratory demonstrated that immuno-modulating agents (α-interferon, cyclosporine A, cyclophosphamide and cortisol) attenuate opiate withdrawal suggesting a possible involvement of the immune system in opiate dependence. More recently, we demonstrated that destruction of the immune system by irradiation also alters the behavioral properties of addiction. The present study was undertaken to determine if visual evoked responses (VER) are altered in three brain regions following destruction of the immune system and if the morphine effects on the VER remain intact after ablation of the immune system. Whole body irradiation to 500 rads selectively ablates the immune system. Irradiation was performed at either 2 hours prior to morphine exposure (pellet implantation), or at 2 hr prior to naloxone injection. VER were recorded from eighteen freely behaving male Sprague-Dawley rats previously were implanted under anesthesia with permanent electrodes. The brain regions chosen were as follows: The superior colliculus (SC) as a control region; the visual cortex (VCx) for a region of higher integrative activity; and the ventromedial hypothalamus (VMH) due to reports demonstrating its interaction with the immune system. The VER's were obtained at 2 hr and 72 hrs following irradiation; as well as 6 days and 9 days post-irradiation to observe any possible subtle alterations not detected at the earlier times. VER's in the SC remained unchanged for the entire time course. The majority of the VER recorded from the VCx remained unchanged at 2 hrs, post irradiation. However, at 3, 6 and 9 days the amplitude of the VER was potentiated. The VER recorded from the VMH was altered at all time points studied. The changes in the VMH and the VCx usually affect the $N_2$ and $P_3$ components of the VER. Finally, the VER following morphine treatments of the irradiated animals were differed from that obtained from intact morphine naive animals. In conclusion, the doses of irradiation used had no destructive effect upon the electrical activity of the CNS as assessed by the VER obtained in the superior colliculus (SC) However, the VER in those CNS areas that have some immune interaction as well as affecting immune function were altered. Moreover, the morphine effects on VER in immunosuppressed animals were differed than those observed in intact animals. These results further support our previous indications of CNS-immune system interaction.

265.3 KAPPA OPIATE MODULATION OF THYROID STIMULATING HORMONE (TSH) RELEASE IN THE RAT. S. Iyengar and P.L. Wood, Neuroscience Research, Pharmaceuticals Division, CIBA-GEIGY Corporation, Summit, NJ 07901.

In rats acute administration of morphine or leu-enkephalin results in decreased TSH secretion (Lomax et al. Neuroendocrinology, 6:146, 1970; Bruni et al. Life Sci., 21:461, 1977; May et al. Horm. Metab. Res., 11:30, 1979). Multiple opiate receptors have been implicated in the release of some pituitary hormones (Pechnick et al. J. Pharmacol. Exp. Ther., 232:163, 170, 1985). However, their role in the release of TSH has not been well characterized.

The effect of various opiates, more specifically kappa opiates, on basal TSH release was studied in the male rat. Plasma TSH was measured (by RIA, using a NIADDK kit) 60 min after acute systemic injections of the drugs. Mu and delta opiates (morphine 16 mg/kg i.p. and DADLE 2 µg i.c.v.) caused significant decreases in plasma TSH, confirming the results of the above workers. These effects were antagonized by naloxone (5 mg/kg i.p. injected twice at 6 min prior to and 30 min after drug) and Win 44441-3 (5 mg/kg i.p. at 10 min prior to drug). Naloxone (2 and 5 mg/kg i.p.) and Win 44441-3 (2 and 5 mg/kg i.p.) by themselves did not have any effect on TSH. The kappa agonists, (-) bremazocine (0.1 and 1 mg/kg), MR 2034 (4 and 16 mg/kg) and U 50488H (8 and 16 mg/kg) caused very significant decreases in TSH (levels were 19 - 25% of controls) that were partially or fully reversed by the antagonists. This response was attenuated in animals made tolerant to U 50488H and challenged with an acute injection of the drug (tolerance was achieved by injecting the drug in ascending order of 10, 15, 23, 34, 55 and 71 mg/kg i.p. every 12 hr). U 50488H had no effect on hypophysectomized animals. Tifluadom, a kappa agonist, also inhibited TSH release significantly.

Thus, 1) Multiple opiate receptors have an inhibitory effect on TSH release, 2) kappa opiates modulate basal TSH release, 3) these studies present evidence for kappa opiate action on yet another pituitary hormone and 4) endogenous kappa opiates may be important mediators of hypothalamic function.

265.4 KAPPA AND MU OPIOID RECEPTOR AGONISTS HAVE DIFFERENT MOTIVATIONAL PROPERTIES AS REVEALED BY PLACE AND TASTE PREFERENCE CONDITIONING R.F. Mucha, M.J. Millan* and A. Herz*. Dept. Neuropharmacol., Max-Planck-Institute for Psychiatry, 8033 Planegg, F.R. Germany, and Addiction Research Foundation, Toronto M5S 2S1, Canada.

Whether the selective activation of individual types of opioid receptors has different motivational effects is not known. Therefore, agonists selective for the kappa and the mu opioid receptor were tested in classically conditioned preference procedures. Such methods give rats a choice between a stimulus paired with drug injections and another paired with vehicle; rats typically prefer stimuli paired with appetitive reinforcers and avoid those paired with aversive stimuli.

Two stimuli, unbiased, place and taste conditioning procedures were used. Naive rats were run on only one method, using three SC injections of drug or vehicle during training and no injections during testing. The kappa agonists produced conditioned aversions: Taste aversions were produced by racemic mixtures of ethylketazocine, tifluadom, and U50-488 and active isomers (+) tifluadom, (-) bremazocine, and Mr 2034. The corresponding inactive isomers either produced no effect or were less potent. Place aversions were produced by U50-488 and (-) bremazocine, but not by (+) bremazocine nor by any of the other kappa receptor agonists tested with the taste procedure. Although naloxone also produces conditioned aversions (place), these were attenuated by arcuate nucleus lesions while the aversions seen with kappa agonists were not. The mu agonists produced conditioned preferences. Place preferences were produced by morphine, fentanyl, and sufentanil. Taste preferences were produced by low doses of these substances; at high doses the taste preferences were absent or replaced by aversions. Inactive isomers, (+) morphine and dextrorphan, did not produce any conditioned preferences, but they did produce conditioned taste aversions.

Therefore, with systemic administration of drug, activation of kappa opioid receptors has aversive properties and activation of mu receptors, appetitive reinforcing effects. This may have implications for understanding the mechanisms of motivation produced by opioid analgesics.

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265.5 ELECTROPHYSIOLOGICAL CHANGES IN SUBSTANTIA NIGRA AFTER DYNORPHIN ADMINISTRATION. A. Lavin* and M. Garcia-Munoz. Centro de Investigaciones en Fisiología Celular, U.N.A.M., P. O. Box 70-600, 04510 Mexico, D.F.

High concentrations of Dynorphin (Dyn) have been measured in the reticular part of the substancia nigra (SNR) (Palkovitz, M. et al, Neuropeptides, 4:193,1984). Recently Dyn cells have been observed in the striatum of cat and rat (Chesselet, M.F. and Graybiel, A.M., Life Sci., 33:37,1983; Zamir, N. et al, Nature, 307:643,1984). The origin and course of the Dyn-containing striatonigral fibres seem to be similar to those containing GABA and Substance P. When injected into SNR, Dyn causes a dose-dependent contralateral rotation which is partially inhibited by naloxone (Herrera-Marchitz, M. et al, Eur. J. Pharmacol.,102: 231,1984). The present experiments were designed to explore the electrophysiological changes induced in SNR by local administration of Dyn. The experiments were performed on 34 male Wistar rats (250-260 g), acutely prepared under Halothane anesthesia. The rats were tracheotomized, fixed to the stereotaxic apparatus and maintained at a constant temperature (37°C). For i.v. injection of naloxone the femoral vein was cannulated. Extracellular potentials were recorded with glass microelectrodes (14-18 Mohms). Dyn was administered by pressure ejection (Picospritzer, 5-20 psi/50 msec) from a glass pipette. Dyn (1-9 Sigma, 50 pM, pH 7.0) and naloxone hydrochloride (DuPont 2.0 mg/kg i.p. or 0.2 mg/kg i.v.) were dissolved in 0.9% saline. Tyrosyl-3,5-(N)-3H-Dyn (1-9, Amersham) was administered in the course of some experiments; radioactivity was determined by liquid scintillation counting.

Twenty-one out of 32 cells recorded in SNR (70%) showed a decrease in firing rate after Dyn administration. Radioactively labelled Dyn was administered in 12 experiments in order to verify the administration of the drug. Radioactivity (486±564 cpm above blank) was observed in 10 of the 12 experiments (83%); 8 cells were in SNR. In 6 experiments once the response to Dyn was observed, naloxone was administered. In every ocassion naloxone antagonized the effect of Dyn, and in most cases it increased firing rate and decreased spike amplitude. In 13 experiments where naloxone was not administered, the duration of the response to Dyn was found to be 6.0±4.5 min. Ten cells were also recorded in SNC; 8 did not respond and 2 were excited.

The results of the present investigation indicate that Dyn exerts an inhibitory effect on SNR neurons which appears to be pharmacologically specific since it is reversed by naloxone.

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265.6 ENKEPHALIN-INDUCED SEIZURES : µ OR δ ?. O.Carter Snead, III, Department of Pediatrics and The Neuroscience Program, University of Alabama at Birmingham, Birmingham, Alabama.

Leucine-enkephalin (LE) and morphine (M) are two opiate compounds which interact with opiate receptors in a specific and saturable manner. Morphine is thought to have a high affinity for µ receptors and to exert its analgesic effect via this opiate receptor subtype while leucine-enkephalin has high affinity for the δ receptor. Both LE and M produce seizures which, although similar in some ways, differ in terms of naloxone dose response and morphology (Snead & Bearden, Neuropharmacology 21: 1137, 1982). This raises the question as to whether δ receptors (Frenk, Brain Res. Rev. 6:197, 1983), µ receptors, or both are involved in the seizures produced by these compounds.

This question has been approached by examining the ontogeny of the EEG and behavioral response to the δ agonist Tyr-D-Ser-Gly-Phe-Leu-Thr (DSLET), as well as D-Ala$^2$-D-Leucine$^5$-enkephalin (DADL). Until recently methodologic difficulties have precluded careful depth recordings and the use of convulsants (e.g. peptides) that do not cross the blood brain barrier in the developing animal. However, using a recently developed technique, rat pups ranging from 1 hour to 84 days of age were implanted with intraventricular cannulae and cortical and hippocampal electrodes (Snead and Stephens, Exper. Neurol. 82:249, 1983). Varying amounts of DSLET or DADL were then administered intracerebroventricularly. (icv). Once a baseline response was obtained, the experiment was repeated in animals pretreated with either naloxone or the specific δ antagonist ICI 154,129. The response of adult animals treated with the latter drug plus leucine-enkephalin was also determined.

DSLET produced spikes and behavioral seizures in adult animals similar to those seen with LE or M. However the ontogeny of DSLET-induced seizures was different from that seen with DADL, LE, M, or the specific µ agent morphiceptin (Snead, Epilepsia 25:662, 1984). DSLET seizures were blocked by both naloxone and ICI 154,129 but LE seizures were blocked only by naloxone.

These data suggest that LE-induced seizures are not δ-specific but may require both µ and δ receptors to manifest themselves.

265.7 PREPUBERTAL DEVELOPMENT OF OPIATE BINDING SITES IN RABBIT BRAIN: CORRELATION WITH LH RELEASE. M. Wilkinson and E.V. YoungLai*. Dept. of Obstetrics and Gynecology, McMaster University, Hamilton, Ontario, Canada, L8N 3Z5.

Hypothalamic opiate peptides may provide an important component of the physiological regulation of puberty. Most studies have been performed in the rat and little is known with respect to the female rabbit. We have recently described the occurrence of a major peak of LH secretion in the immature female rabbit (7th Intl. Congr. Endocr. p. 1555, 1984). This occurs at approximately 35-50 days after birth; puberty normally occurs at about 100 days. In preliminary studies designed to investigate the influence of endogenous opiates on LH release in the rabbit we have quantified the binding of [$^3$H]naloxone (NAL; S.A. 51 Ci/mmole) to opiatergic sites in mediobasal hypothalamus (MBH) and cerebral cortex. Binding assays were performed on brain slices (350μm) as previously described (Brain Res. Bull. 13, 481, 1984). Tissue was obtained from rabbits aged 1, 29, 40, 51 and 100 days. [$^3$H]NAL binding was characterized in cortical slices and was shown to be reversible, stereospecific, saturable, of high affinity and thermolabile. Saturation binding curves were analysed by the method of Zivin & Waud (Life Sci. 30, 1407, 1982). $B_{max}$ values in MBH increased from birth (808±86 cpm/mg tissue; counting efficiency 48%) to a peak at 40 days (3431±358 cpm/mg tissue) after which they declined to 1865±210 (51 days) and 2155±202 cpm/mg at 100 days. In cerebral cortex binding was lower than in MBH (birth: 293±30 cpm/mg) and reached near maximum values by day 29 (838±149 cpm/mg tissue). Receptor numbers did not decline with further maturation. The $K_d$ value, particularly in MBH, showed a similar pattern of change i.e. at birth binding was of high affinity (0.89±0.17 nM) which declined to 3.70±0.50 at day 40 but increased again by day 51 (1.50±0.30 nM).

The major prepubertal peak of LH, which occurs around 35-50 days after birth, appears to correlate with a shift in MBH opiate binding to a lower affinity, higher capacity binding site. Whether this corresponds to a change in the ability of naloxone to release LH remains to be determined.

Financial support was from the Canadian MRC.

265.8 EFFECTS OF β-ENDORPHIN ON ORNITHINE DECARBOXYLASE ACTIVITY: A POSSIBLE ROLE FOR ENDOGENOUS OPIATES IN REGULATION OF TISSUE GROWTH. J.V. Bartolome*, C. Kuhn and S.M. Schanberg*. Dept. of Pharmacology, Duke University Medical Center, Durham, N.C. 27710.

A considerable body of evidence now exists indicating that opiates might participate in growth processes, particularly in the modulation of brain growth. The question remains as to which of the endogenous opiate peptide systems are involved in perinatal regulation of growth processes. The current study examines the effects of β-endorphin on ornithine decarboxylase (ODC) activity in 6 and 25 day-old rats. ODC catalyzes the initial step in the synthesis of polyamines, intracellular messengers which regulate macromolecule synthesis during replication, differentiation and growth (Heby, O., Differentiation, 19: 1, 1981; Pegg, A.E. and McCann, P.P., Am. J. Physiol., 243: C212, 1982). Previous studies have shown that alterations in ODC levels are invariably associated with deficits in tissue maturation and/or function (Slotkin, T.A., Life Sci., 24: 1623, 1979). In the present study, we have shown that intracisternal (i.c.) administration of β-endorphin (1 μg) markedly reduced brain ODC activity in 6 and 25 day-old rats. This ODC response was essentially uniform across brain regions including cerebellum, brainstem + midbrain and cerebral cortex. The inhibitory action of β-endorphin on brain ODC suggest that this endogenous opiate might act as a growth inhibitory factor during perinatal development. Since it has been reported that endogenous opiates might also play an important role in the regulation of growth of peripheral tissues, the effects of β-endorphin on heart, liver and kidney ODC were also investigated. The i.c. administration of β-endorphin resulted in a marked reduction in ODC activity in all three peripheral tissues. In contrast to the inhibitory actions of centrally administered β-endorphin, subcutaneous administration of β-endorphin increased ODC activity in the heart and liver, while no significant changes were observed in kidney ODC. Thus, the reduced tissue ODC levels observed in animals given β-endorphin i.c. appears to be mediated by central effects of β-endorphin. The results from this study demonstrate that β-endorphin can modulate growth-related processes such as ODC and the polyamines, which are necessary for tissue growth and development. (Supported by USPHS Grant No. 2 RO1 MH13688 and RSA Grant No. 2 KO5 MH06489).

265.9 **PROTECTION FROM CEREBRAL ISCHEMIA BY U-50488, A SPECIFIC KAPPA OPIOID ANALGESIC AGENT.** A.H.Tang* and R.C.Silvia* and A.Salvatierra. CNS Research, The Upjohn Company, Kalamazoo, MI 49001.

The kappa opioid receptor agonists have a number of pharmacological properties (e.g. analgesic, sedative, anticonvulsant, diuretic). Because of the implication of opioid receptor mechanism in stroke (e.g. Faden, Stroke 15:575, 1984), we investigated the effects of U-50488, a specific kappa opioid receptor agonist (VonVoigtlander et al., J. Pharmacol. exp. Ther. 224:7, 1983), in several models of experimental cerebral ischemia.

Mongolian gerbils were anesthetized with 2% halothane and the common carotid arteries were occluded bilaterally for 7 or 15 minutes. Seven-minute bilateral carotid occlusion (BCO) produced behavioral hyperactivity 24 hours later (Tang et al., Soc. Neurosci. Abst. 10:126, 1984). There was also selective degeneration of pyramidal neurons in the dorsal hippocampus when examined one week later (Kirino, Brain Res. 239:57,1982). Pretreatment with U-50488E (30 mg/kg i.p.) 30 min prior to BCO protected the gerbils from the post-ischemia behavioral abnormality and prevented neuronal degeneration in the hippocampus. The protective effect on hyperactivity was shared by two other kappa agonists (ethylketocyclazocine and bremazocine), but was partially reversed by the simultaneous administration of naloxone (3 mg/kg). Naloxone (10 mg/kg) given alone had no protective effect. BCO for 15 minutes in gerbils resulted in death for the majority of animals within one week. Pretreatment with U-50488E significantly reduced mortality from 15 minutes of BCO.

BCO in rats of the Fischer 344 strain under brief halothane anesthesia for surgery produced marked cerebral edema in the forebrain within the first few hours. Reperfusion at the end of 4 hours BCO was followed by rapid deterioration and death. Pretreatment with U-50488H (10-30 mg/kg) prior to BCO completely prevented the development of cerebral edema and protected the rats from the lethal effects of BCO in this model. A therapeutic potential for U-50488 in stroke is indicated.

265.10 EFFECTS OF CYCLAZOCINE ON RAT CEREBELLAR PURKINJE NEURONS: COMPARISON WITH PHENCYCLIDINE. M.B. Kim*, K.C.H. Pang*, R. Freedman* and M.R. Palmer. Departments of Pharmacology and Psychiatry, University of Colorado Health Sciences Center 4200 E. 9th Ave., Denver CO 80262 and Denver Veterans Administration Hospital Medical Research Service 1055 Clermont, Denver CO 80220.

Cyclazocine is a benzomorphan which, in addition to more classical opiate properties, binds to the sigma opiate receptor site. Recently, it has been suggested that the sigma opiate receptor is identical to the binding site responsible for the actions of phencyclidine (PCP). Since the electrophysiological actions of PCP have already been demonstrated on rat cerebellar Purkinje neurons, the effects of cyclazocine were also studied in this system with the goal of comparing the electrophysiological effects of cyclazocine to those of PCP. Cyclazocine inhibited the spontaneous firing rates of Purkinje neurons. These responses were stereospecific and qualitatively appeared similar to the effects of PCP. Antipsychotic drugs, haloperidol and fluphenazine, partially antagonized the actions of cyclazocine, suggesting a catecholaminergic involvement similar to the mechanism proposed for PCP. Unlike PCP, on the other hand, the effects of cyclazocine were also partially reversed by the opiate antagonist, naloxone. Taken together, these results suggest that in the rat cerebellum cyclazocine may be interacting with at least two receptor mechanisms: a naloxone-sensitive opiate site, and a naloxone-insensitive site which might involve catecholaminergic mediation similar to the PCP mechanism of action. The naloxone-sensitive effects of cyclazocine, however, may be related to an interaction of the drug with kappa receptors rather than with the more clasical mu or delta opiate mechanisms.

This work was supported in part by USPHS grants AA-05915 and DA-02429. K.P. is a predoctoral fellow (USPHS HD-07072).

265.11 IS INTRATHECAL DYNORPHIN A KAPPA LIGAND IN RATS? C.W. Stevens and T.L. Yaksh*, Departments of Neurosurgical Research and Pharmacology, Mayo Clinic, Rochester, MN 55905.

Opioids produce their effects by an interaction with specific receptors which are classified on the basis of in vivo and in vitro bioassays into subtypes described as mu, delta, and kappa opiate receptors. Previous work in rats chronically implanted with intrathecal catheters has shown that spinally administered delta ligands (e.g. D-Ala$^2$-D-Leu$^5$-enkephalin) inhibit cutaneous, thermally-evoked responses (hot plate, HP; tail flick, TF) and are inactive against visceral, chemical stimuli (writhing test, WT); kappa ligands (e.g. U50488H) are potent on WT but inactive on HP/TF whereas mu ligands (e.g. morphine) are active on all three measures (Schmauss and Yaksh, JPET 228:1-12, 1984).

Numerous in vitro bioassays have established dynorphin A(1-17) and its shorter sequences as potent kappa ligands (see Chavkin et al., Science 215:413, 1982), however in vivo administration has produced controversial results. In an attempt to assess the putative kappa activity of dynorphin A(1-17) and its shorter sequences in vivo, we have examined their effect after intrathecal delivery in rats. We find that dynorphin A(1-17) and (1-13) produced severe motor dysfunction characterized by total loss of hindlimb tonus at doses as low as 10 and 3 nmol/rat, respectively. This motor dysfunction was also produced by dynorphin A(3-13), which is inactive at kappa sites, and was not affected by systemic naloxone (3 mg/kg). Dynorphin A(1-17) and (1-13) at doses below those which produce motor dysfunction had no effect on HP, TF or WT. Dynorphin A(1-7), (1-8), (1-9), and (1-10) had no effect on HP or TF at 30 nmol; and no effect on WT with doses as high as 100 nmol/rat. In contrast, the kappa agonist U50488H (10 nmol) was potent on WT, showed no activity on HP or TF, and produced no motor dysfunction with doses as high as 100 nmol.

| | Minimum Effective Dose (nmol) | | | | | | |
|---|---|---|---|---|---|---|---|
| Dynorphin A Sequence | (1-7) | (1-8) | (1-9) | (1-10) | (1-13) | (1-17) | (3-13) |
| Motor Dysfunction | >100 | >100 | >100 | >100 | 3 | 10 | 30 |

No effect of dynorphin A sequences on HP, TF, or WT at doses just below those producing motor dysfunction.

We conclude that the potent in vitro kappa activity of dynorphin A may not be reflected after in vivo intrathecal administration in rats. Additionally, the motor dysfunction which is seen after intrathecal delivery of the longer chain dynorphins does not appear to be mediated by opiate receptors. (Supported by Grant DA-02110.)

265.12 ELECTROPHYSIOLOGICAL AND BIOCHEMICAL STUDY OF THE ANTAGONISM OF PCP ACTION BY METAPHIT IN RAT CEREBELLAR PURKINJE NEURONS. Y. Wang*, M.R. Palmer, R. Freedman*, M.V. Mattson*, R.A. Lessor*, M.F. Rafferty*, K.C. Rice*, A.E. Jacobson* and B.J. Hoffer, Depts. of Pharmacology and Psychiatry, Univ. of Colo. Health Sci. Cntr., Denver, Colorado 80262; Laboratory of Chemistry, Inst. of Arthritis, Diabetes and Kidney Diseases, NIH, Bethesda, Maryland 20205; and Medical Research Service, Denver Vet. Admin. Med. Cntr., Denver, Colorado 80220.

Recent biochemical reports have shown that metaphit (1-(1-(3-isothiocyanatophenyl) cyclohexyl) piperidine) irreversibly antagonizes about 50% of the PCP receptors in rat hippocampus and striatum. In view of our previous work showing a stereospecific, and therefore presumably receptor mediated, depression of rat cerebellar Purkinje (P) neuron discharge by PCP, we investigated the actions of metaphit in this brain area. Metaphit, applied locally by micro pressure-ejection, irreversibly antagonized PCP-induced slowing of P cells in 19 of 20 neurons tested. This antagonism began 5-20 minutes after metaphit application and lasted up to 90 minutes, the longest time point studied. Metaphit also had a transient direct depressant action. Application of sufficient drug to elicit a marked depressant response was a necessary prerequisite for the subsequent blockade of PCP effects. Little or no antagonism of norepinephrine or GABA-induced inhibitions by metaphit was seen in cells where PCP effects were blocked. Since PCP is an indirect noradrenergic agonist in cerebellum, this finding suggests that the blockade of response is at the noradrenergic nerve terminal, rather than at postsynaptic receptors of P cells. In correlative biochemical studies on cerebellum, metaphit completely inactivated PCP receptors, measured by radioligand binding, in 4 of 6 animals. In the remaining animals, 64% and 32% of the PCP receptors were blocked. We postulate that metaphit is a mixed PCP agonist-antagonist which causes a potent and irreversable pharmacological blockade of PCP actions. This evidence suggests that metaphit could become a useful tool in electrophysiological and biochemical studies of PCP receptor-mediated events.

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265.13 DIURNAL CHANGES IN THE OPIATERGIC CONTROL OF LH RELEASE IN PREPUBERTAL RATS ARE ASSOCIATED WITH DIURNAL VARIATIONS IN HYPOTHALAMIC BUT NOT CORTICAL [3H]-NALOXONE BINDING SITES. W. Jacobson* and M. Wilkinson (SPON: K.B. Ruf). Dept. Physiol. Biophys., Dalhousie Univ., Halifax, Canada B3H 4H7.

Endogenous opiates exert a tonic inhibitory influence on LH release in the rat. The degree of this inhibition is known to change as the animal matures. In addition, sex differences exist as to the degree of inhibitory activity exerted by the opiatergic system at any point in development. It has been demonstrated that in the prepubertal female rat, a clear diurnal variation exists in the LH response to opiate receptor blockade with the opiate antagonist naloxone, the response being maximal in the morning and reaching a nadir in the late afternoon. We present here the results of experiments performed to determine whether this diurnal variation in naloxone responsiveness is the reflection of similar changes in the hypothalamic complement of opiate receptors. Binding studies were performed with saturating concentrations (10 nM) of [3H]-naloxone (NAL) on fresh 400μ thick slices of rat mediobasal hypothalamus (HYP) or cortex (CX) obtained at 0700, 1000, 1300 and 1600 hrs (Method: Brain Res. Bull. 13: 481, 1984). Over this time frame, a clear decrease was seen in the amount of NAL bound to HYP slices (7 am, 27±2 vs 4 pm, 18.1±2.2 fm/mg) but not CX slices (7 am, 10.6±1.1 vs 4 pm, 9.5±1.7 fm/mg) in 26 day old females. There was no change in NAL binding to either HYP (25.4±1.9 vs 25.2±1.2 fm/mg) or CX (11.6±1.2 vs 10±1 fm/mg) slices from 26 day old males over the same time frame. A similar pattern was observed in 30 day old females, with HYP binding dropping steadily over the day, (7 am, 18.5±1.4 vs 4 pm, 7.6±0.8 fm/mg) and CX binding remaining unchanged (7 am, 13.1±0.8 vs 4 pm, 13.3±2.4 fm/mg). In males of this age, however, while CX binding did not show any variation over the period studied, a decrease in HYP binding was apparent by 10 am and persisted through the late afternoon. In 15 day old animals, a somewhat less pronounced decrease in HYP NAL binding was seen in the males, while females showed a transient early afternoon decrease of approximately 50% which returned to early am values by 4 pm. In neither sex did CX binding vary. At 9 days of age, the only tissue in which NAL binding varied over the day was male HYP which showed a decrease from a 7 am high of 20.3±2 to a 4 pm low of 7.2±0.9 fm/mg. These observations may provide a neural substrate for the diurnal variations in responsiveness of LH release to opiatergic influence which is seen in prepubertal rats.

265.14 ANTINOCICEPTIVE EFFECTS OF MU AND DELTA OPIOID RECEPTOR LIGANDS IN THE RAT PERIAQUEDUCTAL GRAY MATTER (PAG) AND MEDULLARY RETICULAR FORMATION (MRF) AS STUDIED BY THE MICROINJECTION TECHNIQUE. T.S. Jensen* and T.L. Yaksh* (SPON: B.F. Westmoreland). Gentofte Hospital, Denmark and Mayo Clinic, Rochester, MN U.S.A.

In rats stereotaxically implanted with microinjection (MI) cannula in either the PAG or the medial/paramedial MRF, microinjection (0.5 μl) of morphine (MOR), sufentanil (SUF), D-Ala$^2$-D-Leu$^5$-enkephalin (DADL) or D-Ser$^2$-Thr$^6$-leucine enkephalin (DSTLE) produced dose-dependent elevations in the response latency on tail flick and hot plate tests. Both mu (MOR and SUF) and delta (DADL and DSTLE) opioid receptor ligands produced a maximal elevation in the supraspinally mediated hot plate response when administered into either the PAG or the MRF. Similarly, mu and delta receptor ligands produced maximum elevations in the spinally mediated tail flick response when microinjected into the PAG. In contrast, delta, but not mu, receptor agonists produced a total blockade of the tail flick response following administration into the MRF. MI of mu (MOR) or delta (DADL) agonists into the PAG or the MRF also inhibition of the visceral chemical evoked writhing response. Systematic examination of the effects of intracerebral naloxone administered into the same site as the opioid ligand revealed a dose-dependent (0.3-5.6 nmol) antagonism of the effects produced by both DADL and MOR. At each dose of naloxone, the magnitude of the inhibition was greater with morphine than DADL on both the hot plate and tail flick. These observations suggest that mu and delta opioid receptor linked systems within the MRF but not the PAG produce their antinociceptive effects by discriminable mechanisms with a differential action on spinopetal versus supraspinally organized modulatory systems.

| MI Site | Tail Flick | Hot Plate |
|---|---|---|
| PAG | | |
| MOR | 3.1 ± 0.01$^+$ | 2.9 ± 0.01$^+$ |
| SUF | 0.2 ± 0.02 | 0.2 ± 0.02 |
| DADL | 0.7 ± 0.05 | 0.5 ± 0.02 |
| DSTLE | 1.4 ± 0.06 | 2.7 ± 0.5 |
| MRF | | |
| MOR | -* | 3.5 ± 0.7 |
| SUF | -* | 0.2 ± 0.01 |
| DADL | 0.1 ± 1.8 | 0.1 ± 1.9 |
| DSTLE | 1.4 ± 0.3 | 1.2 ± 0.2 |

*Submaximal elevation of tail flick latency following intracerebral opioid administration. $^+ED_{50}$ nM.

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265.15 ROLE OF HIPPOCAMPUS IN RAT SHAKING BEHAVIOR. J.D. Connor and B.P. Damiano. Dept. Pharmacology, Hershey Medical Center, Pennsylvania State Univ., College of Medicine, Hershey, PA 17033.

Head shaking behavior has been reported after electrical stimulation of the hippocampal interstitium. The likelihood of current spread from the sites of stimulation has made it difficult to evaluate the extent to which hippocampal cell types actually participate in the response. Our approaches to this issue include: 1) stimulation in awake, unrestrained rats of a synaptic input (perforant path) into the dentate gyrus while monitoring shake behavior and evoked potentials; 2) prelesions of CA3 pyramidal cells to study the need for hippocampal output in the shake response; 3) various drug treatments that evoke shakes in conjunction with field potentials and lesions.

Entorhinal (perforant path) stimulation (1-10 Hz; single, twin or trains of stimuli) in conscious rats caused 5-15 shakes in 2-3 min (higher frequencies caused more shakes). Epileptiform activity in the dentate gyrus was not required for shakes to occur. Stimulation of granule cells directly via the recording electrode elicited responses similar to those produced by entorhinal stimuli. Granule cell discharge as evidenced by a population spike was necessary for shaking responses to occur. Rats with relatively pronounced lesions of CA3 pyramidal cells (kainic acid, 350 ng, bilaterally icv) did not develop shakes with any of the stimulation paradigms. Thus, it appears that entorhinal stimuli cause shakes by activating granule cells which, in turn, initiate hippocampal output.

Met- or leu-enkephalin (200 µg icv) caused shaking behavior (~ 10 shakes/10 min), and epileptiform activity (repetitive spontaneous population spikes and afterdischarges). The enkephalins also decreased granule cell recurrent inhibition, as measured by the twin-pulse technique. Enkephalin disinhibition preceded the epileptiform discharges and shaking behavior, and was prevented by naloxone (10 mg/kg i.p.). Naloxone also blocked shake production. Prior kainic acid treatment blocked enkephalin shakes, but not the depression of recurrent inhibition.

Classical "wet dog shakes" evoked by naloxone in morphine-dependent rats were not associated with changes in granule cell evoked potentials, nor were they affected by kainate pretreatments. Results similar to those produced with morphine abstinence were also obtained with TRH (1 µg icv), a potent shake inducer.

The overall conclusions: 1) synaptic activation of excitatory pathways into and out of the hippocampus causes shaking behavior; 2) some shake inducers (icv enkephalins) act through hippocampal mechanisms; 3) shakes caused by icv TRH or morphine withdrawal apparently require CNS areas other than the hippocampus for their generation or modulation.

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265.16 ANGIOTENSIN-CONVERTING ENZYME INHIBITORS POTENTIATE THE ANALGESIC ACTIVITY OF [MET]-ENKEPHALIN-ARG$^6$-PHE$^7$ BY INHIBITING ITS DEGRADATION IN MOUSE BRAIN. B.S. Barbaz, W. Autry* and J.A. Norman, Neuroscience/Cardiopulmonary Research, Research Department, Pharmaceuticals Division, CIBA-GEIGY Corporation, Summit, NJ 07901

The degradation of the enkephalin-containing heptapeptide Tyr-Gly-Gly-Phe-Met-Arg-Phe (YGGFMRF) was investigated by incubating the peptide with synaptic membranes from mouse brain. The degradation products were derivatized with 4-dimethylaminoazobenzene-4'-isothiocyanate (DABITC) and analyzed by HPLC and by amino-terminal analysis. The incubation of YGGFMRF with synaptic membranes yielded YGGFM and RF as the degradation products. This dipeptidyl-carboxypeptidase activity could be potently inhibited by MK-422 and captopril, inhibitors of the angiotensin-converting enzyme, with $IC_{50}$ values of 8 nM and 95 nM, respectively. The "enkephalinase A" inhibitor, thiorphan, weakly inhibited this dipeptidyl-carboxy-peptidase activity with an $IC_{50}$ value greater than 1 µM.

YGGFMRF, MK-422, captopril and thiorphan all produced a dose-dependent elevation of mouse jump latencies in the hot plate test when administered intracerebroventricularly. However, when sub-analgesic doses of the inhibitor were concomitantly administered intracerebroventricularly with a sub-analgesic dose of the hepta-peptide YGGFMRF, only the angiotensin-converting enzyme inhibitors, MK-422 and captopril, potentiated in the analgesic response of this peptide. These results provide in vitro and in vivo evidence that in the mouse brain, the angiotensin-converting enzyme is the primary enzyme involved in the proteolytic degradation of the hepta-peptide YGGFMRF.

265.17 ENHANCEMENT OF TRYPTAMINE TURNOVER IN MOUSE BRAIN BY MORPHINE ADMINISTRATION. Alice A. Larson. Dept. Veterinary Biology, University of Minnesota, St. Paul, MN 55108.

While tryptamine (TA), a trace indoleamine, is present in the brain, its function in this tissue remains unclear. TA has a rapid turnover rate and is sensitive to changes in the activity of monoamine oxidase. While the spinal C-fiber reflex is inhibited by 5-hydroxytryptophan, a serotonin precursor, it is enhanced by administration of TA (Bell and Martin, JPET 196,373,1976). We have previously reported an enhanced rate of response to the noxious thermal stimulus of the tail-flick assay after intrathecal injection of TA in rats (Larson, Brain Res, 265:109, 1983). More recently, Harrison and Christian (Neurobiology of the Trace Amines, 249, 1983) have found that isolation-induced stress elevates the concentration of TA in brain and adrenal tissue of rats. As TA is thus implicated in pain- and stress-related activities, the purpose of the present investigation is to examine the effect of opiate analgesics on the concentration and turnover of TA in the brain. Endogenous TA was extracted from mouse whole brain homogenates using ethyl acetate, purified using Bio-Rex 70 cation exchange columns and analyzed on an isocratic HPLC system consisting of a C18 reverse phase column and fluorescence detection. The injection of 100 mg/kg of morphine sulfate i.p. 3 hr before sacrifice, or implantation of a 75 mg morphine pellet 72 hr prior to sacrifice did not significantly alter the concentration of endogenous TA. However the turnover of TA in whole brain, as measured by TA accumulation 60 min after administration of pargyline HCl, increases after coadministration of 100 mg/kg of morphine with pargyline compared to pargyline-treated controls. Implantation of one 75 mg morphine pellet at either 48 or 72 hr prior to pargyline also resulted in an enhanced rate of TA accumulation compared to placebo-implanted control mice. Morphine has been found to enhance the activity of a variety of putative neurotransmitters thought to be involved in analgesia, e.g. serotonin and norepinephrine. The present data indicate that both acute and chronic morphine treatment also appear to enhance the metabolism of TA, a trace amine which has been shown to facilitate pain-reflex activity. The enhancement of tryptaminergic activity after morphine treatment may thus be a factor in the development of tolerance to opiate analgesia. This work was supported by NIH Grant NS17407.

265.18 BRAIN SITES OF STEROID-OPIATE INTERACTION IN THE CONTROL OF LUTEINIZING-HORMONE SECRETION IN THE MINIATURE PIG. N. Parvizi*. (SPON:W. Reinhardt). Institut für Tierzucht und Tierverhalten (FAL), Mariensee, 3057 Neustadt 1, F.R.G.

The present study was conduced to study the central sites of action of leu-enkephalin (leu-E) and met-enkephalin (Met-E) on LH-secretion and to determine the steroid-enkephalin interaction.

Twenty adult ovariectomized miniature pigs were bilaterally provided with stainless steel tubings (22 G) bearing a 28 G exchangeable innertubing in amygdala (AMY) and mediobasal hypothalamus (MBH). One week after this surgery and 5 days prior to the beginning of the experiment (EXP) a silastic catheter was inserted into the left jugular vein. All surgeries were performed under stresnil-hypnodil (Jansen, Düsseldorf, FRG) anesthesia. In 3-day intervals, the animals received following treatments: EXP. I:(7 animals)30 ng leu-E or 1 µl saline (0.7 %; solvent) were microinjected (mic) into the AMY, MBH or AMY & MBH. Blood samples (2 ml) were withdrawn from 90 min before to 180 min after the mic in 10 min intervals. Plasma samples were stored at -20° C until LH was measured. One day was asigned as control blood sampling without any mic. EXP. II:(7 animals) was similar to EXP. I except that Met-E was microinjected. EXP. III: the animals (n =6) were microinjected with 3 ng estradiol-17ß (E2) or 30 ng testosterone (T) into the MBH. 180 min thereafter they received a mic of 30 ng Met-E in the same site of MBH. Blood was taken as in EXP.I. In all three experiments each animal received each treatment.

Leu-E decreased plasma LH-levels significantly ($p < 0.01$; ANOVA) when it was given into the AMY & MBH. Microinjection of Met-E into the AMY, MBH or MBH & AMY has no significant effects on LH-secretion. However, there was a pronunced interaction between Met-E and steroids. Microinjection of Met-E 180 min after microinjection of E2 or T results in plasma LH-values which are 30 % and 26 % the levels after the microinjection of E2 and T respectively.

The data indicate that (a) leu-enkephalin may modify the LH-secretion independent of gonadal steroids. Its effect can be brought about by mechanisms involving AMY and MBH. (b) Met-enkephalin amplifies the MBH mediated feedback control of gonadal steroids on LH-secretion.

The work was supported by the German Research Foundation (DFG).

265.19 SUCROSE INHIBITS DISTRESS VOCALIZATIONS AND ELEVATES PAIN THRESHHOLDS IN 10-DAY-OLD RATS: MEDIATION BY ENDOGENOUS OPIOIDS. E.M. Blass, E. Fitzgerald*, and P. Kehoe. Dept. of Psychology, Johns Hopkins University, Baltimore, MD 21218.

The discoveries that: 1) neonatal altricial rats can taste and respond to a variety of substances and, 2) endogenous opioid systems appear to be behaviorally functional in 10-day-old rats led us to evaluate interactions between pleasure, psychological and physiological stress. In one experiment, either 3.5% or 11.5% sucrose or distilled water was infused through jaw cannulae into the mouths of Day 10 pups that were separated from their mothers but group-housed as a litter. Infusions were preceeded by intraperitoneal injections of isotonic saline or naltrexone (0.5 mg/kg b.w.). Paw-lift latencies (PLL) from a 48° C hot-plate were determined either 1 or 3 min after termination of sucrose infusions.

In a second experiment, individually housed rats received through the indwelling jaw cannula infusions of 7.5% or 11.5% sucrose or milk. The number of distress vocalizations (DVs) caused by the isolation was recorded on a minute-by-minute basis for experimental and control isolated pups. Additional groups of rats were so treated following naltrexone injections.

Sucrose infusions markedly elevated PLL of group-housed rats. Latencies increased from 9.2 sec in water-infused animals to 16.1 and 21 sec for rats receiving 3.5% and 11.5% sucrose infusions, respectively. Naltrexone injections reversed the effect as PLL were reduced to control levels. Thus, sucrose infusions very quickly elevated pain threshhold, as inferred by PLL, in non-stressed, group-housed 10-day-old rats.

Infusions of sucrose or milk attenuated the frequency of DVs in isolated rats from a mean of 266 DVs in 5 min to 50, 147 and 112 DVs for 7.5% and 11.5% sucrose and milk, respectively. Naltrexone injections reversed this trend elevating the number of DVs to 223, 280 and 324 DVs per 5 min period for 7.5% sucrose, 11.5% sucrose and milk, respectively. Taken together, these results demonstrate interactions between putative pleasure and pain systems in 10-day-old albino rats. Moreover reversability by naltrexone suggests an opioid-like mediation of these phenomena.

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265.20 OPIOID MEDIATION OF SEPARATION DISTRESS IN 10-DAY-OLD RATS. P.Kehoe and E.M.Blass, Dept. of Psychology, Johns Hopkins Univ. Baltimore, Md. 21218

The present study demonstrates the relationship between distress vocalizations (DVs), analgesia and opioid activity in 10-day-old rats separated from their dam for as few as 5 min. The experimental paradigm consisted of individually isolating 10-day-old pups for 5 min, during which time ultrasonic cries were recorded. The physiological response to the isolation stress was assessed by a hot-plate technique developed to establish a reliable method of measuring neonatal rats' responsiveness to nociception. The number of DVs 5 min and paw lift latencies (PLL) provided the dependent measures. Five groups of pups were studied in the first experiment. Pups tested on the hot-plate immediately after removal from the nest had a mean withdrawal latency of 9s. Rats isolated for 5 min had a latency of 15s and emitted 300 DVs, similar to saline pretreated pups (15s and 320 DVs). Pups that received naltrexone injections prior to isolation emitted 630 DVs during the isolation and exhibited remarkably short PLLs (6s). In contrast, pups pretreated with morphine had a 22s PLL and were relatively quiet (22DVs). Thus, naltrexone increased pain sensitivity and ultrasounds while morphine resulted in the opposite effects.

To determine whether the opioid mediation of these 2 behaviors is central in origin, we replicated the above experiment with solutions administered intracerebroventricularly (ICV) 90 min prior to isolation. Involvement of the endorphinergic system in isolation-stress behaviors was also assessed by pretreating a group with beta-endorphin antibodies (ICV). In general, central injections mimic findings obtained with peripheral treatments. Those isolated for 5 min after no injection or an ICV injection of saline had a mean PLL of 17.9 and 18.2s and emitted 280 and 320 DVs respectively. Morphine given intracranially caused pups to exhibit a withdrawal latency of 30s with 54 DVs. In contrast, naltrexone-injected pups had a PLL of 10.5 and emitted more DVs than any other group (480). Pups administered beta-endorphin antibodies also withdrew from the heat faster than the saline or no-injection control pups (12.3s) but interestingly did not differ statistically from these groups in their DVs (337).

These studies implicate central opioid systems in the modulation of neonatal isolation-induced stress and its expression in ultrasonic calls and reduced pain sensitivity. The beta-endorphin peptide may, in fact, be partially responsible for isolation-induced responses since antibody treatment significantly reduced heat withdrawal latencies relative to untreated isolates but did not significantly change the number of emitted cries.

## SPROUTING AND SPROUTING MECHANISMS

266.1 EFFECTS OF GM1 GANGLIOSIDES ON CHOLINERGIC ENZYMES AND Na-K-ATPase IN THE HIPPOCAMPUS AFTER FIMBRIA-FORNIX TRANSECTION IN RATS. B. Fass, J.J. Ramirez*, S.P. Mahadik & S.E. Karpiak. Psych. Dept., Clark Univ.,Worcester,MA; Neurosurg. Dept., Univ. Virginia Med.Sch; Div. Neurosci., NYSPI, Columbia Univ. Med. Ctr., New York,NY.

To assess whether exogenous gangliosides accelerate the onset of lesion-induced sprouting in the hippocampus (HPC), we evaluated the effects of GM1 treatments on HPC cholinergic enzymes (AChE and ChAT) and Na-K-ATPase (a neuronal plasma membrane marker) after unilateral transection of the fimbria-fornix (FF). Previous research showed that histochemical staining for AChE is intensified and biochemical measures of AChE and ChAT are increased beginning some time between 14-30 days after transection (Gage et al., 1983). These enzyme changes evidently reflect sprouting by a ventral cholinergic afferent to the HPC (Gage et al., 1984).

In the present study, male albino rats (250-400g) were injected with GM1 (10mg/kg,IP) on the day before surgery, the day of surgery and every third day thereafter. The rats were killed at 14 or 18 days after transection, in order to evaluate whether the treatments promoted the onset of cholinergic sprouting in the HPC. AChE staining was virtually eliminated in the ipsilateral HPC, and optical density measures of residual staining were identical for GM1-treated and untreated rats. Biochemical measures of AChE and ChAT were obtained from additional rats killed at 19 days after transection. Enzyme activity was significantly reduced ($p<.001$) in the ipsilateral HPC relative to the contralateral side. Such reductions were greater in GM1-treated rats than in untreated controls ($p<.001$). By contrast, Na-K-ATPase activity in the ipsilateral HPC was increased (+19.2%) in GM1-treated rats and decreased (-18.9%) in untreated controls, relative to the opposite side.

Since AChE and ChAT were not enhanced at 14-19 days after transection in GM1-treated rats, it appears that the treatments did not accelerate cholinergic sprouting in the HPC. This result is consistent with our previous finding that cholinergic sprouting in the dentate gyrus after unilateral entorhinal lesions is not enhanced by ganglioside treatments (Fass & Ramirez, 1984). The observed reductions in AChE and ChAT may signify more rapid removal of degenerating axons, which could be advantageous for terminal growth at later time points. The increased levels of Na-K-ATPase might indicate that GM1 enhances sprouting by noncholinergic fibers in the HPC, and/or "stabilizes" membranes in the HPC (Karpiak, 1983; Karpiak & Mahadik, 1984). It remains to be determined whether these changes reflect a direct effect on enzyme levels/activities per se, or whether GM1 treatments affect the total number of enzyme-containing synapses in the denervated HPC.

Supported by NSF BNS76-17750 (Oswald Steward) and FIDIA contract CS 185-84 (Donald Stein).

266.2 TIME COURSE OF CHANGES IN HISTOCHEMICAL STAINING FOR GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G-6-PDH) IN THE LATERAL SEPTUM AFTER FIMBRIA-FORNIX TRANSECTIONS. D. Stein and B. Fass. Psychol. Dept., Clark Univ., Worcester, MA 01610

Hippocampal efferents project to the lateral septal nucleus (LS) via the fimbria and dorsal fornix (1). Unilateral transections of this pathway denervate the LS and induce sprouting by surviving inputs (2). Sprouting in the LS has been shown to begin at about 6 days posttransection (DPT), proceed maximally until about 25 DPT, and continue until about 120 DPT (3).

In order to assess whether denervation and subsequent reinnervation might affect cellular metabolism, we evaluated the effects of unilateral fimbria-fornix transections on G-6-PDH activity in the LS. Denervation has been found to exert detrimental effects on neuronal metabolism, including reductions in succinate dehydrogenase activity, uptake of 2-deoxyglucose, and incorporation of protein precursor (4,5,6). We were interested in studying G-6-PDH in a model system where lesion-induced sprouting occurs, because previous research showed that the activity of this oxidative enzyme increases during axonal regeneration (7).

Adult male albino rats (300-400g) survived for 2,4,6,8,10,14,18, or 30 DPT and their brains were processed for G-6-PDH histochemistry (after 8). The intensity of staining in the denervated LS was measured with a densitometer and compared with the intensity in the contralateral (control) LS. At 2 DPT, staining was reduced to 61% (±5.2 SEM) of the control value, and at 4 DPT it was 67% (±2.4). The intensity returned to control values by 14 DPT. Thus, metabolic activity in the LS (as reflected by G-6-PDH staining) is dramatically reduced after denervation, and a recovery of activity occurs at the time when reinnervation proceeds maximally (3). The recovery of staining may be due to G-6-PDH within sprouting axons, or it may reflect changes within the denervated/reinnervated neuropil of the LS.

This research was supported by FIDIA contract CS-185-84 (D. Stein). We thank R. O'Connell for use of the densitometer (supported by NINCDS grant NS14453).

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266.3 EMBRYONIC HIPPOCAMPAL TRANSPLANTS INDUCE COLLATERAL AXONAL SPROUTING FROM UNINJURED CHOLINERGIC SEPTAL AXONS Lawrence F. Kromer Dept. of Anatomy & Neurobiology, University of Vermont, Burlington, VT 05405

Prior studies demonstrate that embryonic hippocampal transplants are innervated by regenerating cholinergic septal axons when they are permitted to fuse with the lesioned surface of the septum in an adult recipient (Kromer et al., 1980, Brain Res. 210:153-172). However, it is uncertain whether uninjured cholinergic axons located in the fornix-fimbria are capable of sprouting collateral axons which can innervate a hippocampal transplant if their normal projection to the host hippocampus is intact.

In the present experiments, adult rats received aspiration lesions of the cingulate and parietal cortices overlying the fornix-fimbria at its site of attachment to the posterior septum. A piece of embryonic hippocampus (embryonic day 14-15) was placed on the dorsal surface of the uninjured fornix-fimbria. After a survival period of up to three months the specimens were processed for Acetylcholinesterase (AChE) histochemistry and fast blue retrograde fluorescence tracing in order to determine whether the hippocampal transplants received a cholinergic input. In these experiments the hippocampal transplants formed extensive attachments to the dorsal surface of the host fornix-fimbria and received a dense AChE-positive fiber ingrowth that was directly traced to AChE fibers originating in the fornix-fimbria. In these specimens the normal cholinergic septal-hippocampal projection within the fornix-fimbria appeared to be intact since the host hippocampus possessed a normal pattern and density of AChE fibers. Injections of fast blue into the transplants resulted in the retrograde labeling of neurons in both the medial septum and diagonal band regions.

The present results provide preliminary evidence that embryonic hippocampal transplants are capable of stimulating collateral axonal sprouting from uninjured cholinergic axons in the fornix-fimbria of an adult recipient. Experiments are currently in progress to determine the percentage and distribution of septal neurons that innervate both the transplant and the host hippocampus. This will be accomplished by identifying double labeled septal neurons following the injection of different retrograde fluorescent tracers into the host hippocampus and transplant. (Supported by NIH grant #NS18126)

266.4 SPROUTING OF SEROTONERGIC AXONS IN THE AREA DENTATA AFTER LESIONS OF THE MEDIAN RAPHE NUCLEUS. J.H. Haring. Dept. of Anatomy, St. Louis Univ. Sch. of Med., St. Louis, MO 63104.

The serotonergic (5-HT) innervation of the area dentata (AD) arises from neurons of both the dorsal (DRN) and median (MRN) raphe nuclei, however, the MRN is the primary source of 5-HT fibers, particularly to the dorsal AD. The present immmunocytochemical study has examined the sprouting response of 5-HT axons remaining in the dorsal AD after electrolytic or 5,7-dihydroxytryptamine lesions of the MRN.

In the dorsal AD of unlesioned rats, the distribution of 5-HT immunopositive terminals is most dense beneath the stratum granulosum. Also, a sparse plexus of 5-HT fibers and varicosities is seen in the hilar region and in the stratum moleculare. Two weeks after lesions of the MRN, a severe reduction in the 5-HT innervation is observed in all subsectors of the dorsal AD. Six weeks after MRN lesion, reinnervation appears to be taking place although the density of 5-HT fibers remains less than normal. The hallmark of this 5-HT axon proliferation is the presence of large fibers which originate in the dense 5-HT band of stratum lacunosum moleculare and cross the hippocampal fissure to collateralize and reinnervate stratum moleculare, the region subjacent to stratum granulosum and the hilar region. These studies also suggest that a denervation threshold may exist for the induction of 5-HT axon sprouting. Lesions which result in near total destruction of the MRN induce the appearance of large reactive 5-HT axons. These reactive fibers are less common when fewer MRN neurons are destroyed. Instead, the proliferating axons appear as tangles of 5-HT fibers which expand to fill vacancies adjacent to their normal terminal domaine. These 5-HT tangles have also been observed in the supragranular region of the AD which normally receives little or no 5-HT input. Current qualitative estimates indicate that at least 30-50% cell death must occur in the MRN before the induction of 5-HT axon proliferation will occur.

Long-term anatomical studies of the proliferation of 5-HT fibers after MRN lesion, as well as correlative functional studies using behavioral and electrophysiological methods are being conducted.

Supported by NIH Grant RR 05388 from the BRSG program.

266.5 THE RELATIONSHIP BETWEEN DOPAMINE DEPLETION AND SEROTONIN HYPERINNERVATION IN THE RAT CAUDATE FOLLOWING NEONATAL 6-OHDA TREATMENT.
Towle*, A.C., Mueller, R.A., Lauder, J.M., Breese, G.R. Departments of Anatony, Anesthesiology, Pharmacology and Biological Science Research Center, University of North Carolina, Chapel Hill, N.C., 27514. (SPON:H.Krebs)

Recently, 6-OHDA treatment of neonatal rats has been shown to lead to an increase in the serotonin content of the caudate nucleus. Similar treatment of adult rats does not result in any change of serotonin content. To gain some insight into the mechanism for this hyperinnervation, we sought to investigate the effects of partial depletions of dopaminergic (DA) neurons and fibers on the serotonin innervation of the caudate.

Rat pups were treated on day 5 with either 100, 50, or 25µg 6-OHDA (i.c.v.) and sacrificed 2 months later. Biochemical measurement of the caudate showed that the drug reduced DA levels by 98.3, 80.1, and 72.5% respectively. Serotonin content was increased in the caudate by 45% at the 100µg 6-OHDA and was not significantly changed by the lower doses. Quantitative immunocytochemical analysis was performed to determine if the biochemical changes were reflected by an alteration of nerve terminal density. Rats were perfused with 4% paraformaldehyde, and 10 micron paraffin sections were cut through the caudate and stained for tyrosine hydroxylase (TH) or serotonin immunoreactivity. The number of terminals intersecting the sides of a 0.25mm$^2$ box were counted at 40x. After neonatal 6-OHDA treatment, the density of TH nerve terminals decreased from 237 intersections/mm (saline) to 5.7* (100µg), 55.2* (50µg), and 73.2* (25µg). The serotonergic terminal density was: 18.3 (saline), 45.3* (100µg 6-OHDA), 26.4 (50µg), 24.6 (25µg) (*$p < .05$). The anatomical evidence confirms the biochemical data and suggests that the increased serotonin is due to an increase of terminal density. The observation that incomplete denervation of TH containing neurons does not lead to a partial hyperinnervation indicates that simple competition between DA and serotonergic nerve terminals can not explain the 6-OHDA induced hyperinnervation. Rather the caudate must be virtually depleted of DA before the serotonergic hyperinnervation can occur.

266.6 RECOVERY OF NOREPINEPHRINE (NE) TERMINALS IN SPINAL CORD FOLLOWING NEONATAL 6-HYDROXYDOPAMINE (6-OHDA). K.E. Simmons* and D.J. Jones. (SPON: M.S. Albin). Depts. of Pharmacology and Anesthesiology, The University of Texas Health Science Center, San Antonio, TX 78284

Previous studies in adult rats have demonstrated that destruction of spinal cord norepinephrine (NE) terminals with 6-hydroxydopamine (6-OHDA) produces hindlimb flexor reflex (HFR) hyperresponsiveness. This hyperreflexia also occurs, in time, following transection of the spinal cord and has been attributed to supersensitivity of receptors within the circuitry of the motor reflex arc. In contrast to these results in adult animals, 6-OHDA-induced destruction of spinal NE terminals in neonatal animals does not produce HFR hyperresponsiveness when measured on postnatal day (PND) 15, which is prior to maturation of spinal pre- and postsynaptic NE elements. On PND 45, after neonatal 6-OHDA, HFR hyperresponsiveness was present suggesting that the expression of denervation-induced hyperreflexia requires the maturation of spinal NE postsynaptic receptors.

The present work extended the above studies to evaluate the response of presynaptic neuron activity by measuring the high affinity uptake of $^3$H-NE in upper and lower spinal cord segments from control rats and those treated at birth with 6-OHDA (PND 1 and 2; 100 mg/kg, SubQ). In addition, spinal cords were transected in groups of control or 6-OHDA treated rats on PND 15 (TX-15) or PND 45 (TX-45). These TX animals were maintained until PND 60 at which time they were sacrificed and upper (above TX) and lower (below TX) $^3$H-NE uptake measured. As expected, $^3$H-NE uptake was reduced over 75% in lower cord in adult rats with spinal cords transected on PND 45 (TX-45). However, no difference was noted in upper and lower cord of TX-15 rats suggesting recovery of terminals in the lower cord by the PND 60 sacrifice date. A 75-80% decrease in $^3$H-NE uptake was present in upper and lower cord segments in control, 6-OHDA treated rats sacrificed on PND 60 without TX. Transection of the cord on PND 45 did not alter the depletion produced by the neonatal 6-OHDA since a 60-70% decrease in $^3$H-NE uptake in upper and lower segments was present. In the TX-15, 6-OHDA group, upper cord $^3$H-NE uptake was not different from the TX-45, 6-OHDA group. However, lower cord uptake was similar to the TX-15 control group in which uptake was identical to non-treated controls. These results suggest that following neonatal 6-OHDA, transection of the spinal cord prior to maturation of spinal NE systems induces "sprouting" of the remaining NE fibers to reestablish NE terminal function.

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266.7 SYMPATHETIC INNERVATION OF THE RAT PINEAL GLAND: OVERLAP OF INNERVATION BY THE TWO INTERNAL CAROTID NERVES (ICN) AND SPROUTING FOLLOWING UNILATERAL DENERVATION. J. R. Lingappa and R. E. Zigmond, Dept. of Pharmacology, Harvard Medical School, Boston, MA 02115.

The rat pineal gland receives sympathetic innervation, via the right and left ICN, from neurons whose cell bodies are located in the two superior cervical ganglia. Using quantitative fluorescence microscopy, we have examined the density and distribution of fluorescent profiles in the pineal gland after lesions of the ICN. 10 μm sections of pineal glands were stained using a glyoxylic acid method. Photographs of a portion of every 10th section were analyzed using a grid overlay to determine the density of fluorescent processes. 32 h after sectioning both ICN, by which time the lesioned fibers have degenerated, the density of fluorescent profiles was 3% of that seen in sham-operated controls. 32 h after sectioning one ICN (uniICNx), the density was decreased to 56% of the sham value. Analysis of montages of whole sections indicated that the the magnitude of the decrease following uniICNx was similar in the right and left halves of the sections. Thus the results suggest that each ICN innervates both sides of the pineal gland equally. Zigmond et al. have proposed that the decrease in the nocturnal activity of the enzyme N-acetyltransferase (NAT) that is found on the first night after uniICNx, but not on the second night, is caused by varicosities of the lesioned neurons taking up norepinephrine released by the intact neurons (PNAS 78: 3959, 1981; J. Neurosci. 5: 142, 1985). (NAT is involved in the synthesis of the pineal hormone melatonin.) In order for such "heteroneuronal uptake" to occur (i.e., for terminals of one ICN to take up transmitter released by terminals of the other ICN), the terminal fields of the two ICN must overlap. The anatomical findings presented here suggest the existence of such an overlap.

Sections of pineal glands were also analyzed at longer times after uniICNx. Two weeks after the lesion, the density of fluorescent profiles had increased to 92% of the control value. When the contralateral ICN was cut 2 weeks after the original uniICNx and pineals were removed 32 h later, the density decreased to 2% of the sham value, indicating that all of the increase in fluorescent profiles following uniICNx originates from sprouting of the contralateral ICN and not from regeneration of the lesioned ICN or sprouting and ingrowth of other adrenergic neurons. Interestingly, although nocturnal pineal NAT activity is inhibited 9 h after uniICNx, it is restored to normal by 32h (see references above), that is, before significant sprouting has occurred. It remains to be determined whether the sprouting is important for the restoration of other aspects of normal pineal function. This work was supported by USPHS grants NS 17512 and MH 00162.

266.8 ALTERATIONS IN THE SEROTONERGIC PROJECTIONS TO STRIATUM AFTER DOPAMINE-DEPLETING BRAIN LESIONS IN INFANT OR ADULT RATS. A.M. Snyder, M.J. Zigmond, and R.D. Lund. Dept. of Anatomy and Cell Biology and Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15261.

It has previously been shown that 6-hydroxydopamine (6-HDA) administered to neonatal rat pups so as to produce a near-total destruction of central dopaminergic (DA) neurons results in an increase in the serotonin (5-HT) content of striatum (Stachowiak, et al., Brain Res., 291: 164-167, 1984). Recently, it has been shown that these biochemical changes are accompanied by an increase in the density of 5-HT-immunoreactive fibers in striatum (Mailman, et al., Soc. Neurosci. Abs., 9: 932, 1983), as well as an increase in the number of raphe neurons labelled after HRP injection into striatum (Kaul, et al., Soc. Neurosci. Abs., 10: 1021, 1984). We have now combined immunocytochemistry and retrograde labelling to study this sprouting response in infant- and adult-lesioned rats.

Intraventricular 6-HDA injections in 3 day old rats resulted in the near-total absence of tyrosine hydroxylase-immunoreactive processes in striatum 2-5 months later. A marked increase in the density of 5-HT-immunoreactive fibers was seen in rostral striatum, in confirmation of previous biochemical findings (Stachowiak, et al., 1984). Using retrograde tracing with horseradish peroxidase (HRP) or rhodamine-labelled microspheres, we observed a 40-220% increase in the number of cells in the raphe that projected to the rostral striatum. Subsequent immunocytochemical processing indicated that at least 80-90% of the HRP-labelled cells in the raphe were 5-HT-positive.

Animals given 6-HDA as adults showed no increase in 5-HT-immunoreactive terminals in striatum. In addition, no increase in the number of labelled raphe cells was observed in these animals when HRP was injected into rostral striatum 12-30 days post-lesion, although when HRP injections were made in adult animals 6-12 days post-lesion, an increase in the number of labelled cells was seen. This latter effect may have been due to abortive sprouting, an acute lesion-induced hyperactivity of 5-HT neurons resulting in their taking up more HRP, or a nonspecific effect of 6-HDA treatment.

Our results indicate that deprivation of DA input to the striatum of infant rats leads to a marked and persistent increase in serotonergic raphe projections to rostral striatum, whereas the same manipulation in adult rats only causes a transient appearance of enhanced raphe-striatal labelling. (Supported in part by MH09045, NS19608 and EY05308.)

266.9 SPROUTING OF AN INTACT, UNDAMAGED NEURON: PUTATIVE ROLE FOR CHANGES IN BLOOD OSMOLARITY. D.J. Maetzold and A.G.M. Bulloch. Dept. Med. Physiol., Univ. of Calgary, Alberta, T2N 4N1, Canada.

The freshwater pulmonate Helisoma has been used in a number of studies aimed at elucidating the regulation of sprouting and the formation of electrical synapses. It has been shown that some forms of stress evoke sprouting from a pair of intact buccal ganglia neurons, R5 and L5. Of the stress conditions examined, those involving loss of body fluids, i.e., body wall incision and estivation, were found to evoke sprouting. Furthermore, the observed sprouting followed a particular pattern. A sprouted cell was found to extend a process across the buccal commissure and towards the arborization of the contralateral neuron 5. The current study was undertaken to examine the possible effect of osmoregulatory responses upon neuronal morphology.

Snails were placed in 250ml beakers containing hyperosmotic pond water (20% artificial seawater). Snails were examined at Day 0, 12 Hr., Day 1, 2, 3. Buccal ganglia were dissected, cells R5 and L5 were injected with Lucifer Yellow CH and fixed within 2 hours and prepared for whole mount fluorescence microscopy.

Examination of the neurons R5 and L5 showed that sprouting was maximal (27%) at Day 1. This is in contrast to the earlier study which found a maximal sprouting frequency at Day 3 in response to body incision. The time course of sprouting frequency in stressed animals was as follows: 12hrs.-13%; Day 1-27%; Day 2-12%; and Day 3-11%. The sprout in response to hyperosmotic pond water was similar to that observed in response to injury or estivation. That is, the sprouted cell extended a process across the buccal commissure towards the arborization of the contralateral neuron 5.

In conclusion, the present study supports the hypothesis that sprouting can occur in response to changes in blood osmolarity. This study, however, does not distinquish between the possibilities that the observed sprouting is a direct response to changes in osmolarity or is due to actions of the accompanying change in hormonal balance.

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266.10 PHEOCHROMOCYTOMA PC12 CELL LINE - A LIGHT, SCANNING AND TRANSMISSION ELECTRON MICROSCOPIC STUDY. M.A.England*, M.Gupta and M.F.D.Notter (SPON: E.D.Caine). Depts. of Anatomy, University of Leicester Medical School*, Leicester, U.K. and University of Rochester School of Medicine, Rochester, NY 14642, U.S.A.

Pheochromocytomas are tumors of adrenal medulla with characteristics of immature and adult chromaffin cells. PC12 is a single cell clonal line of these tumor cells and offers a great advantage in understanding the surface and chemical characteristics of these cells in vitro - when actively dividing, and when rendered amitotic. It has been well established that these cells respond to nerve growth factor (NGF) in culture by extending neurites. The present studies were undertaken to investigate the neuronal properties of PC12 cells from a rat clone at the light and electron microscopic level when exposed to (a) antimitotic agents alone, (b) NGF, and (c) a combination of antimitotic agents and NGF. The cells were grown in monolayer cultures on collagen-coated plastic petri-dishes or glass cover-slips. The cultures were divided into four groups - (i) untreated controls, (ii) treated with the antimitotic agents mitomycin C/5-Bromodeoxyuridine(BUdR), (iii) NGF, and (iv) a combination of mitomycin C/BUdR and NGF. At the end of the culture period, the cells from all these groups were processed for fluorescence histochemistry using sucrose-potassium phosphate-glyoxylic acid (SPG) method of de la Torre, scanning and transmission electron microscopy. Growth curves were also plotted after counting cells over a 11 day culture period in the four groups. Our results show that the untreated cells continued to divide and remained unchanged morphologically. NGF treatmant alone inhibited growth during the first few days after treatment but then cells resumed division. Treatment with the antimitotic agents alone inhibited cell division while the cells extended small neurites. The combination of antimitotic agents and NGF inhibited growth while the cells extended longer neurites. With all the treatments the cells showed bright green histofluorescence, indicating the prescence of catecholamines. The results of these studies will be presented at a greater detail.

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266.11 ANTIBODIES TO NERVE GROWTH FACTOR DIFFERENTIALLY AFFECT DORSAL ROOT GANGLION NEURONS IN VIVO. C.E. Hulsebosch, J.R. Perez-Polo+ and R.E. Coggeshall. Marine Biomedical Institute, +Human Biological Chemistry and Genetics, University of Texas Medical Branch, Galveston, TX 77550.

We previously reported a 10-15% increase in unmyelinated dorsal root axons ipsilateral to spinal denervations produced by spinal hemisection or unilateral sectioning of neighboring dorsal roots. This increase in axon number we interpret as sprouting of centrally projecting sensory neurons. In an effort to manipulate the sprouting, 7S mouse submaxillary nerve growth factor (NGF) was presented in excess or endogenous NGF removed by administration of rabbit antibodies to purified beta-NGF (ANTI-NGF). The excess NGF had no noticeable effect. By contrast, ANTI-NGF treatment (3µ l whole rabbit sera/gm.), in hemisected animals increased the number of unmyelinated dorsal root axons by 7% on the operated side and 50% on the unoperated side.

The present study is designed to pursue these findings. Neonatal rats were given daily injections of ANTI-NGF from birth to one month and compared to untreated 1 month old littermates as controls. Below is a table of the means from 8 specimens of the number of myelinated (MY) and unmyelinated (UN) axons in the T4 dorsal roots and the number of neurons in the T4 dorsal root ganglia (DRG) of both groups.

| | ANTI-NGF | CONTROL |
|---|---|---|
| MY | 1595 | 1356 |
| UN | 5944 | 4097 |
| DRG | 3900 | 5884 |

These data show a statistically significant increase in unmyelinated axons ($p<.0001$) and a statistically significant decrease in DRG neuron number ($p<.0001$) in the ANTI-NGF rats. These data indicate that blocking endogenous levels of NGF differentially affects the neuron population in the DRG. Approximately 34% of the neurons require NGF for survival and die if this requirement is not met. The neurons which do survive in the NGF deprived environment sprout even more vigorously than those experiencing surgical denervation.

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266.12 **ARE POLYAMINES INVOLVED IN COLLATERAL SPROUTING?** **C.L. Hix and J.N. Davis. Veterans Administration Medical Center and Dept. of Pharmacology, Duke University, Durham, NC 27705.**

**After removing the input from the entorhinal cortex to the dentate gyrus of the hippocampal formation, many of the other fibers projecting to this area expand their innervation. We have been using the sprouting of cholinergic fibers from the medial septum after a unilateral entorhinal cortex lesion to study the role of polyamines in initiating collateral sprouting. We measured septal ornithine decarboxylase (ODC) activity, the first step in the biosynthesis of the polyamines putrescine, spermidine, and spermine, after entorhinal lesions and determined the extent of sprouting in animals pretreated with α-difluoromethylornithine (DFMO), an irreversible inhibitor of ODC.**

**In the first experiment, male Sprague-Dawley rats received either a unilateral entorhinal cortex lesion or a sham lesion. At various times after the operation, the activity of ODC in the septum was determined. In the second experiment, rats received daily injections of either 0.9% saline or 1 g/kg DFMO in saline for two weeks. A unilateral entorhinal cortex lesion was then made, and the drug treatment continued for an additional two weeks. Rats were sacrificed by perfusing with formalin, and adjacent sections through the hippocampus were stained for either acetylcholinesterase activity (Koelle) or the presence of degenerating nerve terminals (Gallyas silver stain).**

**The results are consistent with the hypothesis that polyamines are not involved in collateral sprouting. We found no differences in ODC activity between sham operated and lesioned animals. Furthermore, DFMO pretreatment did lower brain and renal ODC activity but did not alter the pattern of degeneration or sprouting. Experiments to confirm that the levels of polyamines were lowered by the DFMO pretreatment are currently underway. These data are the first studies of the role of the polyamines in collateral sprouting. Collateral sprouting occurs in uninjured neurons. By contrast, polyamines have been implicated in neuronal responses to injury and in neuronal development. These data suggest that polyamines may be part of the growth and repair processes, but may not play a role in neurite extension, axonal guidance, and neuronal target recognition.**

**(This material is based upon work supported by a National Science Foundation Graduate Fellowship, the V.A., and NS06233.)**

266.13 **INDUCTION OF A NEURITE-PROMOTING FACTOR THAT MAY BE INVOLVED IN THE IN VIVO REACTIVE SPROUTING RESPONSE TO BRAIN INJURY.** D.L. Needels, M. Nieto-Sampedro, and C.W. Cotman. Dept. of Psychobiology, University of California, Irvine, CA, 92717.

In response to partial denervation of a CNS structure, the spared fibers sprout into the deafferented zone. We report here the induction following brain injury or deafferentation of a novel neurite-promoting factor (NPF) that may be responsible for this reactive sprouting response.

A quantitative bioassay was devised in order to measure NPF levels in brain extracts. Purified ciliary ganglion neurons cultured in the presence of high levels of KCl survived for 24 hours, but did not produce neurites. When an extract of rat brain was included in the culture medium, the percentage of neurons with neurites increased in a dose-dependent manner. Quantitative titers were estimated from the dose-response curves.

Brain NPF activity increased several-fold over basal levels following ablation of the entorhinal/occipital cortex. The time course of this response to injury was determined by measuring the activity in tissue surrounding the wound cavity at various times post lesion. The NPF levels reached a maximum between 9 and 15 days post lesion, and subsequently decreased. The rise in neurite-promoting activity closely paralled reactive axon sprouting in vivo. To test whether denervation alone could induce NPF's, we compared NPF levels in hippocampus before and 12 days after electrolytic ablation of the entorhinal cortex. Neurite-promoting activity increased 3.5 fold in the deafferented hippocampus of young adult rats, but did not increase in aged animals. The delay in reactive sprouting in aged animals may be due to a diminished induction of NPF's following denervation.

The injury-induced (but not the basal) activity was sensitive to freezing and thawing, but was reactivated in the presence of thioglycerol or mercaptoethanol. Moreover, all neurite-promoting activity was blocked by treatment with N-ethyl maleimide. These data suggest the presence of a molecule containing an easily oxidized sulfhydryl group that is essential for the neurite-promoting activity in injured-brain extracts. The brain NPF's are probably proteins (based on inactivation by heat or trypsin), but are different from laminin, nerve growth factor, and polyornithine-bindable NPF's.

In conclusion, we describe the induction following brain injury of a protein that may cause reactive sprouting in vivo. Its properties distinguish it from all other reported neurite-promoting factors.

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266.14 THE EFFECTS OF 4-AMINOPYRIDINE AND STRYCHNINE ON RAT SPINAL CORD REORGANIZATION FOLLOWING SCIATIC NERVE LESION. H. Markus* and B. Pomeranz. (SPON: C. THOMPSON). Dept. of Zoology, University of Toronto, Toronto, ONT., CANADA M5S 1A1.

In a previous study (Markus, H. et al., Brain Res., 296: 27, 1984) we investigated the changes that occurred in the rat spinal cord following peripheral nerve lesion. Immediately after sciatic nerve lesion, dorsal horn cells in the lumbar cord (L4 and L5) were deafferented and did not respond to mechanical stimulation of the hindlimb. 21 days after sciatic nerve lesion these deafferented dorsal horn cells responded to mechanical stimulation of the hindlimb. However these responses were not elicited by cutaneous stimulation of their normal hindlimb receptive fields but by cutaneous stimulation of areas of the hindlimb outside their normal receptive fields.

In this study we investigated the time course of dorsal horn reorganization. We observed that the deafferented dorsal horn cells did not respond to mechanical stimulation of the hindlimb during the first two weeks after sciatic nerve lesion. However 2 weeks after the lesion was made the deafferented dorsal horn cells began to respond to mechanical stimulation of the hindlimb.

These results indicate that following peripheral nerve lesions new connections are produced in the spinal cord. Other investigators (Rodin B.E. et al., J. Comp. Neurol., 215: 187, 1983; Seltzer Z. and Devor M., Brain Res., 306: 31, 1984) have shown that new connections in the spinal cord are not due to collateral sprouting but to strengthening of pre-existing but ineffective pathways. To determine whether the appearance of novel receptive fields following chronic sciatic nerve lesion was due to the strengthening of these latent synapses we attempted to activate these ineffective pathways in the acutely lesioned animal by administration of chemicals (4-aminopyridine, strychnine) which facilitate synaptic transmission. Preliminary studies indicate that these chemicals do not consistently cause the appearance of novel receptive fields in the acutely denervated animal.

266.15 PARTIAL DENERVATION OF THE FROG SARTORIUS CAUSES ENHANCED TRANSMITTER RELEASE AT SURVIVING JUNCTIONS.
A.D. Grinnell, Dept. of Physiology and Jerry Lewis Neuromuscular Research Center, UCLA, Los Angeles, CA 90024

Partial denervation of skeletal muscle has long been known to result in terminal and nodal sprouting from surviving axons. This is generally felt to represent a response to signals generated by the denervated muscle and/or degenerating nerve products (Brown, Holland & Hopkins, Ann. Rev. Neurosci., 4:17-42, 1981). In both frogs (Rotshenker, J. Physiol., 292:535-547, 1979) and mammals (Rotshenker & Tal, J. Physiol., 360:387-396, 1985), sprouting from intact motor axons has also been reported following axotomy of contralateral homologous motoneurons. That this sprouting occurs earlier for proximal than for distal contralateral axotomy suggests that it is in response to a signal transmitted between neurons in the spinal cord. In frogs, it has also been shown that contralateral axotomy leads to a long-lasting increase in transmitter release from intact sartorius nerve terminals (Herrera & Grinnell, Nature 291:495-497, 1981). It seems likely that the two contralateral responses are related. If both increased transmitter release and sprouting can be triggered by interaction between homologous motoneuron pools lying on opposite sides of the spinal cord, it might be predicted that similar signals would operate even more strongly within a single motoneuron pool. Thus sprouting in a partially denervated muscle might also be associated with increased transmitter release from intact axons.

To test this hypothesis, frog sartorius nerves were partially sectioned 0.5-1 cm from the point of entry into the muscle, and synaptic strength measured at periods up to two weeks later (prior to reinnervation by sectioned axons.) There is a sharp increase in mean synaptic strength which is evident as early as 6 days after nerve section. The magnitude of the enhancement in synaptic strength is inversely proportional to the number of motor units left intact in the muscle. In this preparation, the increase in release efficacy appears to precede sprouting. It is not yet clear whether this effect on synaptic strength is due to peripheral signals, to transneuronal interaction between axotomized and intact motoneurons, or to altered activity patterns.

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266.16 EFFECT OF EXPANSION OF MOTOR NEURON TERMINAL FIELD ON ACETYLCHOLINE RELEASE. S. Rochel and N. Robbins, Case Western Reserve University, School of Medicine, Cleveland, Ohio 44106.

Herrera and Grinnell (Nature 287, 649 (1980) have reported that transmitter release from frog motor nerve terminals increased when the motor unit size was reduced. Testing the essumption that this relation is reciprocal, we have studied the reverse situation by generating 5 fold increase of the motor unit size.

The efficacy of evoked acetylcholine release was studied in mouse soleus muscle motor nerve terminals at varying intervals after partial denervation produced by L5 nerve section.

Two to four days after section of ventral root nerve L-5, only 2-6 motor units of the normal 21 remained intact, and indirect stimulation evoked only 8-15% of the direct twitch tension.

Indirect twitch tension almost completely recovered between 28 and 50 days after nerve section. However, twitch under lower Ca (0.5mM) and high Mg (1.9mM) was reduced 2 to 3 times compared to contralateral controls. This indicated reduction of evoked transmitter release.

Two subpopulations of nerve terminals evolved in the recovering partially denervated muscles, as observed by intracellular direct measurement of quantal content in 0.4mM Ca, 2.75mM Mg. The quantal content of the first population was greater than contralateral control at earlier times (28-50 days) and equal to control at a later time (90 days). The second population had lower quantal content than control at all times, and probably consisted of newly sprouted terminals, since it was associated with low mepp frequency and slower impulse conduction.

Thus, when the terminal field of the motor units expands 3-5 fold, the efficiency of evoked ACH release is reduced in the sprouted terminals, even 90 days after nerve section. This reduction of efficiency may establish the upper limit for motor unit sprouting. The transient increase of transmitter release in non denervated terminals may reflect either increased axonal transport of synaptic components or greater excitatory drive.
(Supported by NIH A000795 and Fellowship from the Amyotrophic Lateral Sclerosis Association)

266.17 THE ROLE OF MUSCLE FIBER INACTIVITY ON MOTONEURON SPROUTING. M.M. Wines*, S.A. Spector, S.P. Carter*, M.L. TeVrucht*, and M.S. Letinsky. Ahmanson Laboratory of Neurobiology, Jerry Lewis Neuromuscular Research Center, University of California, Los Angeles, CA 90024.

Motoneurons supplying striated muscles in amphibians and mammals are seen to sprout unmyelinated outgrowths from nodes of Ranvier and endplate arborizations following a variety of experimental perturbations. Muscle fiber inactivity produced by intramuscular injection of botulinum toxin or $\alpha$-bungarotoxin, or following nerve block with tetrodotoxin results in prolific amounts of motoneuron sprouting. Since each of these substances alters the physiological status of either the axon or its associated muscle fiber, it is unclear if the inactivity produced is the definitive stimulus to sprouting.

In a recent study by del Castillo and Escalona de Motta (J. Cell Biol. 78:782, 1978) the topical application of formamide was seen to selectively eliminate muscle fiber contractile activity without altering presynaptic release mechanisms or myofiber electrical activity. Given these qualities, following formamide application motoneuron sprouting can be assessed in an environment devoid of muscle contractile activity in which the physiological state of the neuromuscular junction has not been altered.

The cutaneous pectoris muscle (CP) of adult frogs (Rana pipiens) was unilaterally exposed to 4M formamide for an initial incubation of 15 minutes. This produced complete paralysis of the CP muscle for 4-5 days. With repeated exposure to formamide it was possible to block all muscle fiber activity up to 28 days. Prior to each application the muscle was stimulated indirectly to ensure that complete inactivity had been maintained. Visualization of the nerve terminals was accomplished by staining with tetranitroblue tetrazolium. The morphology of the presynaptic neurites remained unchanged until day 19, afterwhich moderate amounts of terminal sprouting were noted. The frequency of this terminal sprouting from synaptic arborizations increased with time, whereas nodal sprouting was never observed.

The induction of motoneuron sprouting by formamide is interesting in that it is initiated over a much longer time course as compared to the onset of sprouting following the application of other neurotoxins (e.g., 4-6 days after botulinum toxin). It appears that CP motoneurons have the inherent ability to sprout well before day 19 as significant amounts of terminal sprouting was observed as early as 7 days following partial denervation of this muscle. The disparity between the onset of sprouting after formamide versus other toxins suggests that inactivity alone is not the immediate cue responsible for sprouting, and may further suggest that motoneuron sprouting following each of these methods is the response to two or more distincty different stimuli.
Funded by NIH grant NS13470 and The Easter Seals Societv.

266.18 LONG-TERM SURVIVAL OF THE ISOLATED GOLDFISH MAUTHNER AXON. S.J. Zottoli, L.E. Marek, M.A. Agostini and S.L. Strittmatter. Department of Biology, Williams College, Williamstown, MA 01267.

CNS axons of certain invertebrates, when isolated from their somata, are known to survive both morphologically and physiologically for extended periods of time. These isolated segments can play an important role in the regenerative process, providing pathways along which regenerating axons find their targets. Although morphological studies have indicated survival of isolated axonal segments of vertebrate CNS neurons, no physiological studies have been reported. Utilizing the goldfish Mauthner cells (M-cells), we report morphological and physiological survival of isolated axons for at least 2 months.

Fish 8.5 to 11.5 cm in body length were maintained between 13.5-15.0°C and kept on a 12 hr light-dark cycle. The M-axons were injured by crushing the brain at the spinomedullary level. For morphological studies, the brains and spinal cords of 7 fish were fixed and processed for electron microscopy at postoperative intervals from 27-95 days. The M-axons were studied in spinal cord segments 3.5 cm distal to the M-cell somata, since the M-axon diameter decreased in a proximodistal direction in the 5 control fish studied. The wound site was sectioned to confirm M-axon injury. For electrophysiological studies, KCl electrodes (3-10MΩ) were used to intracellularly stimulate M-axon distal segments and muscle activity was recorded with a pair of stainless steel wires placed in the mid-trunk region of the fish. Lucifer yellow was injected into the distal segments to confirm that the M-axons had been severed.

M-axons appeared "normal" on EM examination for up to 77 postoperative days. The area of the M-axon sheath did not change during this interval as compared to controls. Between 87-95 postoperative days, the M-axons had degenerated with only myelin debris to mark their former position. Intracellular stimulation of 7 M-axons in 5 fish between 47-62 postoperative days resulted in ipsilateral EMGs in all cases.

Thus, M-axons survive up to 77 days after separation from their somata and are able to conduct action potentials and initiate muscle activity for at least 62 days. The mechanisms for survival and the role of these isolated segments may play in the regenerative process of the M-cell or any other CNS neuron are currently not known. However, the accessibility of the M-axon provides a unique opportunity to answer such questions.

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267.1 OCTOPUSES AND SQUIDS FOR DEVELOPMENTAL STUDIES NOW AVAILABLE THROUGH LABORATORY CULTURE. J.W. Forsythe* and R.T. Hanlon* (SPON: P.M. Adams). Marine Biomed. Inst., Univ. Texas Med. Br., Galveston, TX 77550.

Cephalopod molluscs are advanced invertebrates that have been used extensively by the biomedical research community, particularly in Europe. Unpredictable availability of live animals has limited their use in the U.S., but we have recently cultured 5 species of Octopus (Hanlon, R.T. and Forsythe, J.W., Lab. Anim. Sci. 35:33, 1985) and the squids Loligo forbesi and L. opalescens (Yang, W.T. et al., Aquaculture 31:77, 1983) through the life cycle (cycles range 6-15 months). In vivo and developmental studies are now possible. Cephalopods are the only invertebrates possessing both a closed circulatory system and a highly vascularized brain in which the lobes are separate, rendering them good comparative models to vertebrates. Examples of recent experimental uses include: the glial blood-brain barrier; photo-transduction in the vertebrate-like eye; morphogenesis, pattern formation and regeneration of neurally controlled chromatophores in the skin; and aging (single breeding with optic gland hormone initiating both maturation and senescence). Octopuses are quite hardy, easily transportable, and can be maintained at inland aquaria. They are particularly useful in learning and memory experiments (even hatchlings are adaptable to training paradigms), they have short- and long-term memory stores, they narcotize easily and can withstand operations. Squids are renowned for their giant axons and giant synapses, which are models for nerve transmission, axonal transport and cell membrane biophysics. Synaptic transmission at the neuromuscular junction of the squid chromatophore organ is also being actively studied (Florey, E. et al., Comp. Biochem. Physiol., in press). Recent development of a semi-intact squid preparation lasting up to 4 hours (Dubas, F. et al., J. Exp. Biol., in press) will enhance future physiological experimentation. Ontogenetic studies have not been possible until now, but limited numbers of laboratory-cultured squids and octopuses are now available to selected researchers.

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267.2 SEROTONIN-MEDIATED CHANGES IN SENSORY INPUT TO THE JUVENILE CRAYFISH ESCAPE RESPONSE CIRCUIT: DIFFERENCES CORRELATED WITH REARING CONDITIONS. R.A.Fricke, Dept. of Anatomy, Emory University School of Medicine, Atlanta, GA 30322.

Serotonin has been found to produce a variety of effects in Crustacea. In the periphery, serotonin increases transmitter release at certain neuromuscular junctions (Glusman and Kravitz, J. Physiol., 325:233, 1982), while in the CNS it increases the firing rates of postural flexor motoneurons (Livingston et al., Science, 208:76, 1980) and suppresses sensory transmission in the escape response circuit (Glanzman and Krasne, J. Neurosci., 3: 2263, 1983).

We recently found that escape responses of juvenile crayfish are not inhibited by restraint (Fricke and McDonald, unpublished). In adult crayfish restraint decisively inhibits escape tailflips (Krasne and Wine, J. Exp. Biol., 63:433, 1984). To determine if the absence of restraint-induced inhibition in juveniles reflects the delayed development of postsynaptic serotonergic mechanisms, I have recorded sensory EPSPs in an escape command cell, the lateral giant interneuron, using animals having a body length of 1-2 cm. Serotonin superfused over the semi-intact juvenile nervous system at concentrations of $10^{-4}$ to $10^{-3}$M produced a variety of effects on sensory EPSPs triggered by electrical stimulation of abdominal ganglion 2nd roots. In 5 cases EPSPs were inhibited (% reduction = 45.5% ± 14.5% SD). This is qualitatively similar to previous observations in adults. On the other hand, EPSPs were substantially augmented (% increase = 93.8% ± 36.3 SD) in 4 other cases, and in 1 case EPSPs were not significantly changed during exposure to serotonin, but showed marked augmentation (44% ± 25%) after returning to normal crayfish Ringer. EPSPs recorded from different segmental lateral giants of any one animal were similarly affected by serotonin.

This unusual serotonin-mediated augmentation of sensory input to the escape circuit was accompanied by inhibition of spontaneous activity in postural flexor motoneurons recorded extracellularly from abdominal ganglion superficial 3rd roots. Thus, there appears to be a coordinated reversal of serotonin actions that affects locomotor and postural systems of the crayfish abdomen. Although the cause of these changes is uncertain, there was a strong correlation between the social environment in which animals were maintained and the cellular actions of serotonin. Augmented responses were seen in animals (4 of 5) that were maintained individually in small (4" x 4") plastic containers, whereas inhibition was seen in most animals that had been maintained communally (5-25 animals), in larger (7" x 13") plastic containers.

267.3 ENVIRONMENTAL REGULATION OF BEHAVIORAL DEVELOPMENT IN THE CRAYFISH. R. Toler* and R.A. Fricke (SPON: P. Lennard). Dept. of Anatomy, Emory Univ. Sch. of Med., Atlanta, GA 30322.

During early postembryonic development crayfish pass through a stage during which characteristic modulations of adult escape behavior are not evident. It has been proposed that early inflexibility of escape behavior depends on the existence of a strong monosynaptic sensory input to the escape command cell (the lateral giant interneuron) and that command cell growth results in the partial decoupling of this electrotonic synaptic connection (Fricke, Br. Res. 332:139, 1984). To further explore the relationship between growth of the lateral giant interneuron (LG) and the development of escape response plasticity we have: (1) determined more precisely the stage at which escape plasticity appears; and (2) characterized lateral giant growth changes during the course of postembryonic development.

We manipulated the growth rates of siblings by subdividing broods and raising these groups at two different temperatures (18.2-19.8° C) and by varying the frequency of feeding (1/week and 3/week). We found that plasticity of escape behavior (habituation and restraint-induced inhibition) appeared when crayfish reached a body length of 3-4 cm, regardless of their age.

We used intracellular injections of horseradish peroxidase and standard histological techniques to characterize lateral giant growth during postembryonic development. We also measured conduction velocity and input resistance as additional indices of lateral giant size. In the smallest animals studied (1-2 cm) LG has a very simple and compact dendritic arbor, axon diameter is less than 10 µm and input resistance is 10-20 x $10^6$ ohms. At maturity, LG axon diameter is 90-100 µm, the dendritic tree is more complex (e.g. more higher order branches) and input resistance is 100-500 x $10^5$ ohms. Initially the increase in LG size and complexity parallels overall body growth. Parameters reflecting LG growth, however, plateau beginning when crayfish reach a body length of 3-4 cm, the point at which behavioral plasticity is first noticed. This substantial increase in LG size and the matching decrease in LG input resistance are compatible with the hypothesis that partial decoupling of electrotonic sensory inputs results from an increased postsynaptic electrical load that is unmatched by an increase in monosynaptic sensory connections.

267.4 CENTRAL PROJECTIONS OF PERIPHERAL SENSORY CELLS LACKING FUNCTIONAL ACTIVITY IN DROSOPHILA MOSAICS. M. G. Burg and C.-F. Wu. Dept. of Biology, Univ. of Iowa, Iowa City, IA 52242.

Two temperature-sensitive paralytic mutations of Drosophila, $para^{tsl}$ and $nap^{ts}$, are implicated in affecting sodium currents, blocking axonal conduction at high temperatures. The $para^{tsl}$ $nap^{ts}$ double mutants are completely lethal even at 17°C (Nature 286:184, 1980). Previous results showed that it is possible to produce small-patch mosaics containing a single double mutant bristle sensory cell. Behavioral studies confirmed that the $para^{tsl}$ $nap^{ts}$ sensory cell is non-functional at all temperatures tested (17-25°C), while the rest of the nervous system functions normally. Further EM examination of the non-functional $para^{tsl}$ $nap^{ts}$ sensory cell showed normal mechanoreceptor ultrastructure (Soc. Neurosci. Abstr. 10:513, 1984).

It is interesting to know whether lack of activity may affect pathfinding and formation of central projections of these sensory cells. We examined the morphology of the identified bristle sensory cells located on the dorsal mesothorax using the cobalt backfilling technique. Backfilling showed that each identified cell had a characteristic projection pattern, with only minor variations occurring between flies. Results demonstrated that $para^{tsl}$ $nap^{ts}$ non-functional sensory cells follow a normal pathway, project into the proper region of the thoracic ganglia, and form branching patterns typical of normal sensory cells. Moreover, no major differences were found between the central projection patterns of $para^{tsl}$ $nap^{ts}$ sensory cells when compared to the contralateral control projections within the same fly. Therefore, functional activity appears to have no direct effect on the pathfinding and arborization of projections of these sensory cells. Supported by NIH grants NS 00675, NS 18500, and a grant from Searle Scholars Program to C.-F. Wu, and by NIH Pre-doctoral Genetics Traineeship GM 07091 to MGB.

267.5 ALTERED DEVELOPMENT OF THE DROSOPHILA GIANT FIBER PATHWAY IN THE TEMPERATURE-SENSITIVE MUTANT SHIBIRE: CONTROL BY TIMING OF HEAT-PULSE. M.R. Hummon and W.J. Costello. Dept. Zool. and Biomed. Sci./Col. Osteo. Med., Ohio Univ., Athens, OH 45701.

The giant fiber (GF) pathway in Drosophila can be investigated by stimulating the brain and recording from target muscles in the thorax. Minimum latencies of 0.81 msec for TTM (jump muscle) and 1.25 msec for DLM (wing depressors) indicate GF activation in wild-type (Tanouye & Wyman, 1980, J. Neurophys. 44:405). Minimum latencies of shibire (shi) flies reared at the permissive temperature (18-22C) were 0.97 msec for TTM (9 flies, n=20) and 1.45 msec for DLM (9 flies, n=24). Shi flies exposed to 6h heat-pulse (HP) of 30C during early pupal development show heat-sensitive periods and anomalous cuticular development (Poodry, et al., 1973, Devel. Biol. 32:373); as well, the GF motor system is altered by a fusion of the 6 DLM fibers into 3 (Costello & Salkoff, 1983, Neurosci. Abstr. 9: 832) and by disruption of the PSI (peripherally synapsing interneuron) connection to DLM motor axons (Hummon & Costello, 1984, Neurosci. Abstr. 10:1032).

To examine the existence of critical periods of development in GF pathway components, shi flies reared at 22C were given HP at 1-66h of pupariation (~1-44h @ 25C). Minimum latencies for DLM were higher ($\alpha < 0.05$) in all HP groups with survivors (HP onset 16-66h; HP @ ≤15h was lethal) and synchrony of right and left DLM was usually absent. Minimum latencies as high as 1.82 msec were measured (HP @ 18,36,38h). Minimum latencies for TTM were occasionally higher ($\alpha < 0.05$) than non-HP for test groups with HP <48h.

Morphology was investigated with serial 5 μm sections for light microscopy and selected serial thin-sections for TEM. Wild-type morphology of the GF pathway (King & Wyman, 1980, J. Neurocyt. 9: 753) occurs in non-HP shi flies, with GF extending past the cross-over of PSI in the mesothoracic ganglion to reach the TTMn, and PSI readily detectible in the peripheral nerve, PDMN. Flies with HP onset at 16-18h lack a prominent PSI cross-over in CNS, lack GF extending past this area, and lack well-defined PSI in PDMN. PDMN in such flies has a higher number (~200) of small axons (diameter, 0.2-0.3 μm) than in non-HP or HP @ 66h flies (~100). Morphology of flies with HP onset at 66h is variable; 2 of 3 studied showed normal 6 DLM and 1 of 3 had entirely normal PSI morphology in CNS and PDMN. PSI cross-over was not clearly identifiable in 2 flies; only one had any trace of PSI in PDMN. It is possible that this period of development is close to the endpoint of GF pathway development.

GF pathway components and target muscles are differentially altered as a function of timing of HP. These differences may be a function of their differential timing in development. Thus, shi will permit us to investigate the critical periods of development of neuronal components and targets and interactions necessary for proper pathway formation.

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267.6 DEVELOPMENT OF GIANT FIBER PATHWAY COMPONENTS IN DROSOPHILA: POSSIBLE INVOLVEMENT OF COATED VESICLES. W.J. Costello and M.R. Hummon. Dept. Zool. and Biomed. Sci./Col. Osteo. Med., Ohio Univ., Athens, OH 45701.

Because of its genetic repertoire, Drosophila melanogaster can be used to great advantage in studying development. We have been engaged in looking at the development of components of the giant fiber (GF) pathway by using the temperature-sensitive mutant shibire (shi). When exposed to non-permissive temperatures (<28C), shi flies become paralyzed due to a failure in synaptic transmission. Recent work (Costello & Salkoff, 1982, Neurosci. Abstr. 8: 494; Kosaka & Ikeda, 1983, J. Cell. Biol. 97:499) has indicated that the shi defect is in the recycling of membranes, specifically the "pinch-off" mechanism in endocytosis.

We have found that by heat-pulsing (HP) shi pupae at particular stages of development, various anomalies in the GF pathway are induced. These anomalies can occur in neurons and muscles alike. Thus, we can 1) prevent normal GF connectivity to targets in the CNS such as TTMn (jump muscle motoneuron), 2) prevent the PSI (peripherally synapsing interneuron) from forming its normal pathway out a peripheral nerve (PDMN), 3) affect normal connectivity of PSI with the motor axons of the DLM (wing depressor muscle), 4)prevent normal boundary formation of the DLM such that the muscle consists of 3 fused muscle fibers instead of the normal 6 muscle fibers (Costello & Salkoff, 1983, Neurosci. Abstr. 9:832; Hummon & Costello, 1984, Neurosci. Abstr. 10;1032; 1985, Neurosci. Abstr. 11: in press; in preparation).

During initial formation, these components interact with each other. For example, in the early pupa, such interactions are readily seen to occur between adult myocytes and larval muscles serving as their end targets. Adult myocytes send out many fingers which connect with and project into the larval muscles. Many transient junctions are also formed between the myocytes and larval muscle and between nerves and larval muscle. As well, numerous coated vesicles/pits are present in the larval muscle, forming opposite myocytes or nerve. These coated structure are prevalent only during this period, which corresponds to the same period at which a HP would prevent normal boundary formation in the DLM. Before or after this critical period, such structures are absent.

Coated vesicles are an indicator of receptor-mediated endocytosis and play a role in intercellular trafficking of molecules. Since the shi defect is affecting such membrane recycling processes, we can disrupt normal development arbitrarily with HP. By disrupting this process during critical periods of development, normal formation of the GF pathway components is prevented. It thus seems likely that the coated vesicles are playing a role in disseminating molecular information to developing/degenerating cells regarding pathfinding and target interaction.

Supported by Muscular Dystrophy Association.

267.7 THE DEVELOPMENT OF SEXUALLY DIMORPHIC NEURONS IN THE GENITAL DISC OF DROSOPHILA MELANOGASTER. B. J. Taylor*and W. A. Harris. (SPON: G. L. Harris). Biology Dept., UCSD, La Jolla CA 92093.

In order to ask how genes known to control sexual differentiation in Drosophila melanogaster, act on sexually dimorphic neurons, we have begun to characterize the development, differentiation, and central projection patterns of sensory neurons derived from the genital disc. Horseradish peroxidase (HRP) fills of adult flies show distinct central projections in the abdominal ganglion for female versus male bristles. Antibodies raised against HRP ($\alpha$-HRP) appear to bind specifically to a cell surface marker expressed soon after differentiation (Jan & Jan, 1982). Using a monoclonal $\alpha$-HRP antibody (provided by Yuh Nung Jan), we have stained genital discs from male and female larvae and pupae.

The genital disc is bilaterally symmetrical and contains the separate primordium for the female and male genitalia and a single anal primordium. Depending on the chromosomal sex of the tissue, only one of the genital primordia develops and the anal primordium undergoes either male or female differentiation. In genital discs from either sex, the first neurons to become immunoreactive are found around pupariation in the sexually expressed genital primordium but not in the repressed primordium. In the male, the first neurons are associated with the presumptive lateral plate and clasper regions of the disc. Neurons associated with the anal plates appear by 4-8 hours after pupariation. In the female, the first neurons appear to be associated with the internal genital primordium and slightly later neurons are found in the external genital primordium and presumptive anal plate region. Thus, while the first neurons have the same temporal onset of differentiation in the two sexes, the location of the neurons are in sexually dimorphic regions of the genital disc.

Several genes affect the differentiation of sexually dimorphic somatic tissues. Mutations in two of these sex-determining genes tra, transformer, and tra-2, transformer-2, convert a chromosomally female fly into a male one. The normal $tra^+$ and $tra\text{-}2^+$ gene products are needed throughout the development of sexually dimorphic tissues for the proper differentiation of female characteristics. A temperature sensitive mutation of the tra-2 locus has made it possible to separate the decisions for the number of bristles from their morphology (Belote & Baker, 1982). We are extending this analysis to the sensory neurons found in the genital disc and analysing the role that tra and tra-2 play in neuronal differentiation.

Supported by the NIH.

267.8 DEVELOPMENT OF HOMOLOGOUS SEROTONERGIC NEURONS IN OPISTHOBRANCH MOLLUSKS. A.J. Longley (SPON: P. Grant). Pacific Sciences Institute, Box 835, Friday Harbor, WA 98250, and Friday Harbor Labs.

Gastropod mollusks possess neurons which are uniquely identifiable; some of these cells have been recognized as homologous in several orders. I have examined patterns of serotonergic (5HT) neurons in Phidiana (Hermissenda) crassicornis and several related opisthobranchs using an antibody specific for serotonin in ganglion wholemounts. Using the same antibody (also in wholemount), I have followed the development of 5HT neurons in Phidiana and some of its relatives.

In adult Phidiana, Aeolidia papillosa, Armina californica, and Tritonia diomedea, the homology of several neurons was clearly indicated by shared transmitter, location, and axon distribution. The serotonergic giant cerebral neuron (GCN), with axon branches in the lip nerve, buccal ganglion, and buccal nerves, was present in all species examined. In addition to this well characterized neuron, an unpaired left pedal neuron, with the principal branch of its axon in the left pleural nerve, was also found in each species. This neuron appears to be LPdl, identified as 5HT containing in Tritonia (Audesirk et al., Comp. Biochem. Physiol. 62C:87-91, 1979). In Hermissenda, the axon branching and soma location of this neuron correspond to that of LPdl studied by Jerussi and Alkon (J.Neurophysiol. 46:659-671, 1981). 5HT containing somata are absent from the buccal and pleural ganglia.

During development, in very early veligers of Phidiana, 5HT immunoreactivity is first seen in fine, varicose axons in the region of the central ganglia before the somata for these axons have appeared. The GCN somata appear at the time of statocyst formation. At this stage, each GCN extends a varicose axon toward the velar lobes and across the midline at the velum, suggesting that the GCN's innervate the velar lobes before they are lost at metamorphosis. By metamorphosis, serotonergic processes have extensively innervated the region of the buccal ganglion and LPdl can be seen extending its axon in a loop, apparently through the pleural ganglion. The smaller serotonergic cerebral and pedal neurons of the adult are not yet visualized with the antibody shortly after metamorphosis. Early development of 5HT neurons has also been followed in Aeolidia and Melibe leonina; in all species, the GCN's appear at the same stage and extend axons toward the velum and across the midline.

The similarity of early development of these homologous neurons indicates that the developmental mechanisms which give rise to these neurons have been conserved. Currently, other antibodies are being used to distinguish other metabolic classes of nerve cells in adults (RD Longley and AJ Longley, Neurosci. Abst., 1985) and during development of related species. It is now feasible to compare, at the cellular level, evolutionary differences in nervous system development.

267.9 HOT AND COLD SHOCKS PERTURB THE SYNAPTIC ORGANISATION OF FLY PHOTORECEPTOR TERMINALS. A. Frohlich and I.A. Meinertzhagen, Department of Psychology, Dalhousie Univ., Halifax, N.S., Canada

The rules governing synapse formation between identified neurons of the lamina, the first optic neuropile of the fly's compound eye, have been investigated further with special reference to the synapses of the photoreceptor terminals. Six receptor terminals (R1-6) provide input to a cartridge, the unit module of the lamina. Each receptor forms a population of equivalent, uniform synaptic contacts. At each contact an invariable combination of four postsynaptic elements is assembled. These are: two monopolar interneurons (L1, L2) and, at proximal depths, two processes of an amacrine cell. In order to test the combinatorial rules of assembly of these elements we have perturbed the developing visual system, so as to affect the availability of postsynaptic elements.

Flies, Musca domestica, were subjected to cold or hot pulses (5h at 4°C; 1h at 37°C) during the first half of normal pupal development at 20°C, before synaptogenesis occurs. The flies were prepared for electron microscopy at emergence. Survey montages and series of 40-100 sections were analysed. The effects were qualitatively similar for hot and cold pulses, and also for exposure at different developmental stages. They included: in the retina, the frequent loss of R7 and less frequent loss of one or more cells R1-6; in the lamina, the less orderly arrangement of elements, greater variability in cartridge receptor terminal complement and occasional supernumerary postsynaptic cells. We have examined in detail several cartridges with such augmented composition, including one "mega"-cartridge with 4 L1, 2 L2 and 14 receptor cells (the latter are distinguishable ultrastructurally, while L2 is distinguished from L1 by its feedback synapses upon R1-6). The analysis reveals that all receptor terminals are equivalent in the formation of gap junctions and synapses. Rules of postsynaptic combination are strictly adhered to. L1 always pairs with L2 as two elements of a synapse. Receptor terminals may form synapses with more than one L1 or L2, while one L1 may pair with more than one L2 at different synapses, and vice versa. It therefore seems that cartridge affiliation plays no role in synaptogenesis. Rather, neuron classes only must be recognised, all neurons of a given class being equivalent. The results simply the existence of neuronal class labels but the absence of cartridge labels at the time of synapse formation.

Supported by NIH grant EY-03592.

267.10 METAMORPHOSIS OF A CPG: REUSE AND SUBSEQUENT MODULATION OF THE LARVAL ECDYSIS MOTOR PROGRAM FOR THE GENERATION OF ADULT-SPECIFIC ECLOSION BEHAVIOR. K.A. Mesce and J.W. Truman. Dept. of Zoology, Univ. of Washington, Seattle, WA 98195.

Metamorphosis of the hawkmoth, Manduca sexta, is marked by dramatic changes in both body morphology and neuronal organization. Many of these neuronal changes underlie stage-specific behaviors such as crawling in the larva, the defensive gin-trap response in the pupa, and flight in the adult. Ecdysis behavior, or the shedding of the old cuticle, is one behavior, however, that persists throughout metamorphosis and is periodically expressed as the insect molts from one stage to the next. Larval, pupal, and adult ecdyses are all triggered by the same neuropeptide, eclosion hormone, which acts on the CNS to evoke the respective motor patterns. Previous work (Weeks & Truman, 1984, J. Comp. Physiol. 155:407) showed that the larval ecdysis behavior involves an anterior-directed, peristaltic wave of contractions accompanied by the transient retraction of segmental prolegs as each segment is drawn forward. At the pupal stage, the behavior appeared quite different because the prolegs were absent, however, the motor pattern generated by the pupal CNS was essentially identical to that seen in larvae, including the firing phase of motor units that formerly controlled the prolegs.

The major movements shown by the adult during its ecdysis involve a rapid retraction of the abdominal segments in a metachronous fashion followed by their extension coupled with wing muscle contractions. These movements are very different from those shown by larvae and pupae and suggested that the larval CPG was dismantled during metamorphosis and a new one constructed for the adult stage. However, when descending inputs to the adult abdominal ganglia were blocked, the abdomen lapsed into a motor pattern which was identical (on both behavioral and electrophysiological criteria) to that shown at larval ecdysis. Thus, the larval ecdysis CPG appears to be conserved through metamorphosis whereupon new descending inputs modulate the output of this CPG to produce the different behavior displayed by the intact adult. Various aspects of this modulation will be discussed.

267.11 MONOCLONAL ANTIBODIES AGAINST THE SEXUALLY DIMORPHIC OLFACTORY SYSTEM OF *MANDUCA SEXTA*. A. Hishinuma†, S. Hockfield§, R. McKay§, and J.G. Hildebrand†. †Dept. Biol. Sci., Columbia Univ., New York, NY 10027, and §Cold Spring Harbor Lab.,Cold Spring Harbor, NY 11724.

Olfactory receptor cells in the antenna of the sphinx moth *Manduca sexta* send their axons through the antennal nerve (AN) into the ipsilateral antennal lobe (AL) of the brain. The primary olfactory fibers terminate in the neuropil within glomeruli, where they make synaptic contacts with AL neurons. Among other classes of sensory cells, receptors specialized to respond to the sex pheromone of the conspecific female moth are present exclusively in male antennae. The axons of these sexually dimorphic olfactory cells all project to a male-specific neuropil region in the AL, the macroglomerular complex (MGC), where they make synapses with male-specific AL neurons. Axons of mechanosensory neurons at the base of the antenna in both sexes join the intracranial segment of the AN and project into the brain.

As part of continuing studies of the functional organization and postembryonic development of the antennal sensory pathways in *Manduca*, and of the molecular basis of neuronal diversity, we have generated monoclonal antibodies (MAbs) against the male system. Mice were immunized with homogenates of ALs and intracranial segments of ANs from adult male moths, and supernatant media from cultures of hybridoma cells prepared by standard methods were screened immunohistochemically on sections of male ALs. Of the interesting hybridoma lines we have successfully cloned, five secrete MAbs that are cell type-specific, including a male olfactory-specific MAb.

*Olfactory-Specific Antibody* (OSA) stains olfactory receptor cells in both males and females. Cell bodies, dendrites, and axons of the primary olfactory neurons stain with OSA, but no other elements in the brain, subesophageal ganglion, or prothoracic ganglion bind this MAb. Using electron-microscopic immunocytochemistry (EMI) with HRP-conjugated secondary antibody, we have shown that OSA recognizes a surface antigen on olfactory cells and their axons. Axons of OSA-immunoreactive cells are fasciculated together in the AN. *Male Olfactory-Specific Antibody* (MOSA) stains cell bodies, dendrites, and axons of a subset of receptor cells in males but *not* in females. In the AL, MOSA labels the male-specific MGC but not the "ordinary" glomeruli common to both male and female ALs. *Mechanosensory Antibody* stains the antennal mechanosensory fibers but not the primary olfactory axons. *Glial-Specific Antibody* selectively stains glia in the AN and AL, as well as glia elsewhere in the CNS. *Synapse-Specific Antibody* (SSA) stains glomeruli (the sites of all known synapses in the AL) but not other AL regions lacking synapses. EMI shows that SSA reacts with some but not all synaptic terminals in glomeruli, suggesting that these labeled terminals are contributed by a subpopulation of AL neurons. Current efforts aim to characterize the antigens of these MAbs and to explore their expression during adult development.

267.12 CONTRIBUTIONS OF SENSORY AXONS AND CENTRAL NEURONS TO THE DEVELOPMENT OF GLOMERULI IN THE ANTENNAL LOBES OF *MANDUCA SEXTA*. K.S. Kent* and J.G. Hildebrand. Dept. Biol. Sci., Columbia Univ., NY, NY 10027.

As part of our continuing studies of the postembryonic development of the antennal lobes (ALs) in the brain of *Manduca sexta*, we have examined the projections of primary sensory axons and the morphology of central neurons, which normally have arborizations in the glomeruli of the AL, under conditions in which the sensory axons are prevented from reaching their normal target sites or reach them via atypical routes.

Sensory axons from the labial "pit organ" (LPO) project into the subesophageal ganglion (SEG), forming two tracts that bifurcate and project bilaterally to a single glomerulus (LPOG) in each AL [Harrow et al., *Soc. Neurosci. Abstr. 9*:216, 1983]. Antennal sensory axons enter the AL and project to all of the other glomeruli. Our methods of anterograde staining of afferents with cobalt reveal no antennal sensory projections to the LPOG. To examine the intrinsic capacity of sensory fibers to form discrete knots resembling glomeruli, we prevented antennal or LPO axons from reaching the AL by cutting the antennal nerve, removing the brain, or cutting the circumesophageal connectives prior to the ingrowth of adult sensory axons. Antennal fibers formed a neuroma in the head, and axons from the labial palps were restricted to the SEG. To test the ability of sensory axons to seek their normal targets and to examine the specificity of the axons for particular target sites, we redirected antennal or labial axons by severing the antennal nerve or transplanting the anlage of the labial palp to the antennal socket on the head. Antennal axons often entered the brain via an atypical route, and axons from the labial palp entered the brain without traversing their normal tracts in the SEG. Finally, to study the capacity of central neurons to form discrete glomerular arborizations in the AL in the absence of normal sensory inputs, we removed anlagen of the antennae or labial palps prior to metamorphosis.

Our findings show that: (a) afferent axons from the antenna and the LPO form knot-like terminal projections even when prevented from reaching their targets in the AL; (b) sensory axons can reach their target sites in the AL via alternate routes and remain specific for their respective target sites; (c) sensory fibers can project to target sites of their contralateral counterparts if prevented from reaching their normal ipsilateral targets or forced to grow into the contralateral side of the brain; and (d) central neurons deprived of sensory input maintain dense arborizations in "protoglomerular" regions of neuropil in the deafferented AL that correspond to, but are less regular and discrete than, normal glomeruli. Thus we believe that afferent axons and central neurons develop certain characteristic morphological profiles independently of the presence of each other. The development of discrete, fully formed glomeruli in the AL, however, depends upon interactions between sensory axons and central neurons.

267.13 EARLY EVENTS IN THE DEVELOPMENT OF THE ANTENNAL LOBES OF THE BRAIN OF THE MOTH MANDUCA SEXTA. Leslie P. Tolbert. Dept. of Anatomy, Georgetown Univ. School of Medicine, Washington, DC 20007.

The antennal system of the moth Manduca sexta arises and develops during postembryonic development and offers an accessible system in which to study cellular morphogenesis and synaptogenesis. As shown by Sanes and Hildebrand (Dev. Biol. 51:282 & 300), the antennal imaginal disk everts at the onset of metamorphic adult development, and sensory neurons are born. The sensory neurons extend axons to innervate the forming antennal lobes (ALs) of the brain. A previous study of the histological development of the AL and of the maturation of AL synapses during the latter half of adult development (Tolbert et al., J. Neurosci. 3:1158) indicated that many important cellular events occur during the first half of development. The current study focuses on the early development, with the long-term goal of understanding the developmental interactions between the antennal sensory neurons and the neurons and glial cells of the AL.

The mature AL comprises two clusters of AL-neuron cell bodies, and a central area of large, criss-crossing neurites surrounded by an array of dense, compact glomeruli, where the synaptic interactions between antennal sensory axons and AL neurons occur. Glomeruli are separated from each other by almost-complete envelopes of glial processes. On day 2 of the 18 days of metamorphosis, before the earliest antennal sensory axons have reached the AL, the lobe is a mound of light-microscopically homogeneous neuropil, ringed by a tightly packed layer of glial cell bodies and surrounded by cell bodies of AL neurons. As sensory axons begin to reach the AL, the outer region of neuropil becomes dense and compact. A few glial processes begin to infiltrate the condensed neuropil. By day 5, glial cells form irregular septa subdividing the condensed neuropil into regions that by day 6 are light-microscopically identifiable glomeruli. In our earlier study, we had found the first evidence of synapses at day 6; the synapses had small, weakly staining membrane-associated densities and a small number of vesicles in the presynaptic element, and thus looked immature. With improved ultrastructural preservation, I now find clear examples of synapses as early as day 2. The synapses look like mature synapses, with darkly staining membrane-associated densities and hundreds of vesicles in the presynaptic element. Since the synapses appear before the influx of antennal sensory axons, they are presumed to be between AL neurons. I am presently looking at the initial formation as well as the ultimate fate of these synapses in both normal ALs and ALs that are never allowed to receive antennal sensory axons.

These findings indicate that the AL is more highly differentiated at early stages than previously recognized.

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267.14 IMMUNOCYTOCHEMICAL REACTIVITY OF NEURONS IN WILD-TYPE AND MUTANT C. ELEGANS TO ANTISERA AGAINST GABA, SEROTONIN, AND CCK. S. McIntire* and R. Horvitz (SPON: E. Wolinsky), Dept. of Biology, MIT, Cambridge, MA 02139.

The nematode Caenorhabditis elegans is unique in that the complete cell lineage from fertilized egg to adult has been determined and found to be essentially invariant among individuals. This organism's small size (1 mm) and rapid life cycle (4 days) have facilitated extensive genetic analyses. Furthermore, the morphology and connectivity of each of the 302 neurons in the adult have been determined from reconstructions from electron micrographs of serial sections. We have identified reactivity of certain neurons in C. elegans to antisera raised against GABA (gamma-aminobutyric acid), 5-HT (serotonin), and CCK(cholecystokinin). We have identified behavioral mutants abnormal in anti-GABA staining patterns; these mutants define eight genes involved in neurotransmitter expression and/or neuronal morphogenesis.

The antibody to GABA stains the ventral and dorsal nerve cords, a series of commissures, and 19 cell bodies of the ventral nervous system. The number and locations of these cells and processes indicate that they are the DD and VD motorneurons. A set of four neurons, possibly the RME motorneurons, located immediately anterior to the nerve ring also stain. Additional putative GABAnergic neurons are present in the heads and tails of both hermaphrodites and males.

The antibody to 5-HT labels the HSN motorneurons, the pharyngeal NSM neurosecretory motorneurons, a set of six male-specific ventral cord motorneurons (either CA3-CA8 or CP3-CP8), as well as other unidentified neurons in the heads of both hermaphrodites and males and the tails of males.

The antibody to the neuroactive peptide CCK labels cells that have been tentatively identified as the hermaphrodite-specific VC motorneurons and the male-specific CA3-CA8 or CP3-CP8 motorneurons. The male-specific CEM sensory neurons as well as several additional unidentified neurons in the head and tail also appear to stain.

By screening existing "uncoordinated" mutant strains for abnormalities in staining patterns with anti-GABA antisera, we have identified eight genes that can affect GABA expression, the positions of GABAnergic cell bodies, and/or the growth of GABAnergic axonal processes.

267.15 LOW TEMPERATURE REVERSIBLE NERVE BLOCK INITIATES CLAW TRANSFORMATION IN ALPHEID SHRIMPS. DeF. Mellon and G. Cox*, Dept. of Biology, Univ. of Virginia, Charlottesville, VA 22901.

Alpheid shrimps (snapping shrimps) exhibit remarkable morphological plasticity in that the asymmetry of the first pair of thoracic legs can be reversed in adult animals (Wilson, E.B., Biol. Bull. 4: 197, 1907; Darby, H.H., Pub. Carnegie Inst. Wash. No. 435: 347, 1934). Assymetry reversal is normally triggered by loss of the larger "snapper" claw, and it involves transformation of the remaining, smaller "pincer" claw into a snapper, and the concomitant regeneration of a new pincer at the site of the original snapper. We have shown previously that transformation of the pincer can also be triggered by severing both nerve trunks in the existing snapper claw (Mellon, DeF., Stephens, P.J., Nature, 272: 246, 1978). This leads to a situation of symmetry, with snapper claws on both sides. We now report that pincer transformation is also triggered by maintaining the snapper claw at ambient temperature below 6°C for a period of 5-6 days. At this temperature, nerve impulse activity is blocked and thus constitutes a possible causative agent in the onset of transformation. We currently lack sufficient data to determine whether, at these ambient temperatures, axoplasmic transport is also blocked.

When both claws are surgically removed at the same time, both regenerates reproduce their original condition. We are now cooling the pincer claw for periods of 5-6 days following snapper removal. The results will indicate whether cold-blocked nerve in an otherwise transforming animal can influence the expression of claw type on the opposite side. These studies will be important in elucidating the trophic mechanism by which peripheral nerve controls claw type in crustaceans.

267.16 MUSCLE FIBER TRANSFORMATION IN SNAPPING SHRIMP:EVIDENCE FOR FIBER DEGENERATION. K.M.Mearow and C.K.Govind, Dept. of Zool., Scarborough Campus, University of Toronto, West Hill, Ontario. M1C 1A4

The dimorphic claws of the adult snapping shrimp (Alpheus sp.) exhibit an interesting plasticity, in that if the major snapper claw is autotomized the remaining minor pincer claw will transform into a snapper. In the pristine state, the pincer closer muscle is composed of a central band of fast fibers sandwiched by slow, while the snapper muscle has all slow fibers. During claw reversal, the pincer fast fibers gradually transform to slow, on the basis of sarcomere length (Stephens and Mellon, 1979, J.Comp.Physiol. 132:97).

We wished to determine the time course of transformation of another muscle fiber property, i.e., the myofibrillar ATPase activity, using enzyme histochemistry. Thus, snapping shrimp were induced to autotomize the major and then at appropriate periods later, the transforming claw was removed and examined histochemically.

Our results suggest that the fast fibers degenerate, rather than change to slow during claw reversal. At several days prior to the first molt after autotomy, there was still a distinct band of fast fibers in the transforming pincer. However, by the second molt, the fast fiber band had completely disappeared, although the space where it had been was still evident. By three molts there was no longer any space left and the muscle was completely slow. The disappearance of the fast fiber band suggested muscle degeneration during the intermolt between the first and second molts. In order to confirm this, the transforming muscle was examined with EM. The central region showed the presence of some rather abnormal appearing fibers. These were in various states of disorganization, ranging from areas with intact short sarcomeres to total loss of fibrillar integrity; phagocytic cells were also seen. These disrupted fibers observed with their attachments to the central tendon were adjacent to normal appearing long sarcomere fibers. Further EM examination of transforming claws is being carried out to determine the time course of the fast fiber loss. However, on the basis of present results, it would appear that fiber degeneration and replacement, rather than fiber transformation, occurs during claw reversal in snapping shrimp.

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267.17 ROLE OF NERVE IN DETERMINING CONTRACTILE PROTEIN ISOFORM EXPRESSION IN CRUSTACEAN MUSCLE. M.M. Quigley*, G. Cox* and DeF. Mellon, Jr. Dept. of Biol., University of Virginia, Charlottesville, VA 22901.

Differentiation of muscle fibers into mature fast and slow forms involves selection of appropriate contractile protein isotypes. It is known that in vertebrates nerve activity has a profound effect in determining myofibrillar protein expression. In contrast to most vertebrate muscle, adult crustacean fibers exhibit polyneuronal innervation, often with both phasic and tonic axons supplying a single fiber. How this heterogeneous innervation influences gene expression in its target muscle fiber is not known. The main claw closer muscles ($Cl_1$) of the snapping shrimp, Alpheus, offer a unique system for investigating neural influences in crustacean muscle fibers. The first thoracic legs consist of a large snapper claw and a smaller pincer. The main closer muscle of the snapper is composed entirely of slow fibers, while the pincer $Cl_1$ contains both fast and intermediate fibers. Two exciter and two inhibitor axons distribute terminals in different combinatons to the muscle fiber population in each claw. It has been shown previously that the pincer $Cl_1$ contains a single TnT isoform which can be distinguished from snapper TnT by SDS-polyacrylamide gel electrophoresis. We have used this difference to test the role of nerve in initiation of the quasi-developmental transformation of the pincer claw into a snapper. If claw transformation is hormonally regulated, then it may be expected that the process could occur in the absence of an intact nerve supply to pincer $Cl_1$. To test this idea efferent axons to pincer $Cl_1$ were cut during claw transformation. After two molts TnT isoform expression was assayed in the closer muscles of experimental animals and compared to that of normally transforming animals. The results indicate that an intact nerve supply to the transforming claw is necessary to see a change in TnT expression.

To further investigate the role of nerve in determining isoform expression we have raised monoclonal antibodies against myosin purified from slow muscle (snapper closer). Four antibodies have been identified which show differential reaction with myosin from fast and slow muscles. Characterization with ELISA, a western blotting of high resolution-low percentage acrylamide gels and immunocytochemistry now allow us to conclude that there is a minimum of five MHC isoforms in Alpheus striated muscle. It is our goal to use these highly specific markers to correlate protein isoform expression with the patterns of innervation seen in the claw closer muscles.

267.18 MONOCLONAL ANTIBODIES THAT DISTINGUISH AMONG AXON TERMINALS OF IDENTIFIED MOTOR NEURONS IN THE COCKROACH. J.L. Denburg, R.T. Caldwell*, J.A Morrison*, Biology Deptartment, Univ. of Iowa, Iowa City, IA 52242

In the nervous system of the cockroach, Periplaneta americana there are identified motor neurons that have the ability to selectively reinnervate their target leg muscles following axotomy. It was previously demonstrated that among the set of 6 coxal depressor muscles (CDMs) studied in detail each muscle innervated by different motor neurons could be distinguished by its cell surface glycoproteins. The question addressed in this study is whether there are detectable molecular differences among the identified motor neurons. Hybridoma techniques are ideal for these studies.

Mice were inocculated with thoracic ganglia of adult insects and hybridomas were produced by the usual procedures. Supernatants of hybridoma cultures were screened for monoclonal antibody (MAb) binding to frozen sections of thoracic ganglia and leg muscle. The extensive branching of the motor neuron axon within the muscle and the multi-terminal nature of the innervation ensured that every section through a muscle would contain many neuronal processes.

These procedures enabled the isolation of 28 MAbs that selectively bind to sections of ganglia and to nerve branches within sections of muscle. Most of these MAbs bind with equal intensity to nerve branches in all of the CDMs. However, of particular interest are two MAbs that selectively bind to axon terminals in some of the muscles and not others.

MAb 6E7 binds to axon terminals in only 2 of the 6 CDMs. From the known pattern of innervation of these muscles, this would indicate that these axons are from inhibitory neurons or from the dorsal unpaired median cells which modulate contraction of these muscles. MAb 1216E10 binds to axon terminals only in those 4 of the 6 CDMs that receive innervation from motor neuron $D_S$. The absence of binding of either of these MAbs to neuronal somata makes it difficult to definitively identify the neuron synthesizing the antigen. However, the neuronal location of these antigens is indicated by the loss of MAb binding to sections of muscle that had been maintained in a denervated state for two weeks. The antigens of both MAbs are membrane-bound and their localization can be observed in whole mounts of muscles. This enables the visualization of all the axon terminals in each of the muscles.

These results indicate that there are molecular differences between axon terminals of identified motor neurons that undergo a specific cell recognition process when reinnervating muscles during axonal regeneration. (Funded by NIH grant NS 14295)

267.19 A MUTANT DROSOPHILA MOTOR NEURON: ANATOMY AND REDUCED HRP UPTAKE. D.H. Baird and M.S. Hillis*. Department of Biology, Yale University, New Haven, CT 06511.

We are studying the giant fiber (GF) pathway in mutant and wild-type flies to approach the question of synaptic specificity. We have further characterized a motorneuron of the pathway, the tergotrochanteral ("jump") muscle motor neuron (TTMMN), using HRP. A tungsten needle is dipped in concentrated HRP solution, and inserted through the dorsal insertion of the TTM. The histological procedures used to stain the HRP containing neuron are a modified version of those used by Koto (1983, Ph.D. Thesis, Yale). We concur with Koto's findings, concerning the anatomy within the ganglion, of the wild-type adult TTMMN. Peripherally, the TTMMN enters the muscle at a point about 1/3 of the TTM's length (ventral to dorsal). Before the axon enters, it forks. Both branches extend among the muscle fibers (one dorsally, one ventrally) and bifurcate further. Near the ganglion midline, the medial branch (MB) of the cell usually curves anteriorly overlapping the distal bend of the GF axon. Nomarski optics were used to visualize the GF.

Thomas and Wyman (1984, J. Neurosci. 4:530-538) isolated two non-jumping mutants, pas and ben which disrupt the GF-TTMMN synapse. We find in pas flies that the distal portion of the MB is reduced in length and/or diameter compared to wild-type. This finding is especially interesting considering that the synapse between GF and TTMMN (made by this branch, King and Wyman, 1980, J. Neurocytol. 9:753-770) is physiologically abnormal in pas flies (Thomas and Wyman, 1984). Pas flies carrying a chromosome deficient for the pas locus [Df(pas)] show reduced HRP staining in both the percentage of animals with stained TTMMNs, and the intensity of staining. 50% (n=34) of the flies of the genotype pas/Df(pas) showed the entire TTMMN stained while 92% (n=50) of wild-type TTMMNs stained completely. Because we see even, rather than granular staining, we believe HRP diffuses into this neuron through damaged endings as opposed to uptake in vesicles. It is possible that there is a general reduction in HRP uptake due to reduced numbers or size of neuromuscular endings. This is unlikely because of the normal muscle potentials of the TTM when its motor neuron is directly stimulated. The reduced staining may be due to reduced transport of HRP, either generally or only in the MB. The MB is less darkly stained than the PB in 4 flies carrying two different deficiencies with a small region of overlap in or near the pas locus (near polytene chromosome band 19E5). This observation suggests that reduced tranport in the MB correlates with the absence of a normal GF-TTMMN synapse in flies which may be completely deficient for the pas locus. [Support: NS-07314 to R.J. Wyman]

268.1 CHRONIC CYSTEAMINE ENHANCES ANTAGONIST-INDUCED DOPAMINE RECEPTOR SUPERSENSITIVITY. D.B. Lozovsky, D.C. Jimerson and I.J. Kopin. NINCDS and NIMH, NIH, Bethesda, MD 20205

Some recent studies suggest that enhanced prolactin secretion mediates the development of antagonist-induced dopamine (DA) receptor supersensitivity (Hruska, R.E., et al., Eur. J. Pharmacol. 65: 455, 1980; Life Sci., 30:547, 1982), whereas others have disputed this mechanism (Jenner, P. et al., Eur. J. Pharmacol. 76:31, 1981; Borison, R.L. and Diamond, B.I., Pharmacologist, 21:268, 1978; Gordon, J.H., and Diamond, B.I., J. Neurochem., 42:523, 1984). We have studied the effect of cysteamine - a potent depletor of immunoreactive pituitary and plasma prolactin - on antagonist-induced DA receptor sensitization. Rats were injected with haloperidol (1 mg/kg daily, i.p.) or domperidone (5 mg/kg, twice per day, i.p.) alone or in combination with cysteamine (50 mg/kg, twice per day, i.p.) for 21 days. The effect of cysteamine alone was also studied. Control rats received i.p. injections of vehicles. On the 24th day the rats were decapitated and striata removed and frozen. The number of DA receptors in each striatum was determined by measuring the specific binding of [$^3$H] spiperone (1.2 nm) to striatal membranes. The affinity (Kd) of the binding was calculated from Scatchard plots of the data obtained for pools of striatal membranes. In rats treated with haloperidol or with domperidone, [$^3$H]spiperone binding was significantly increased as compared to controls (31.5 ± 1.5 pmole/g tissue, 29.4 ± 0.8 pmole/g tissue and 25.1 ± 1.0 pmole/g tissue, respectively). Chronic treatment with cysteamine resulted in additional significant increases in the numbers of DA receptors (36.4 ± 0.9 pmole/g tissue and 33.3 ± 1.6 pmole/g tissue for haloperidol - and domperidone-treated rats, respectively). Cysteamine alone had no effect on [$^3$H]spiperone binding in vehicle-treated rats (23.1 ± 2.0 pmole/g tissue). None of the treatments affected Kd of the [$^3$H]spiperone binding. These results provide indirect evidence that prolactin may attenuate drug-induced sensitization of striatal DA receptor, which is consistent with results obtained in hypophysectomized rats by Gordon, J.H. and Diamond, B.I. (J. Neurochem., 42:523, 1984). Other possible mechanisms for the cysteamine effect observed in this preliminary study include an effect on tissue neuroleptic levels or an action mediated through other neuroendocrine alterations, e.g., decrease of somatostatin levels.

268.2 **AMPHETAMINE HAS MULTIPHASIC EFFECTS ON ADENYLATE CYCLASE ACTIVITY IN STRIATAL AND MESOLIMBIC RAT BRAIN REGIONS** J. M. Roberts-Lewis and M.E. Gnegy, Departments of Psychology and Pharmacology, University of Michigan, Ann Arbor, MI, 48109.

Amphetamine has complex, dose-dependent, multiphasic, effects on many measures of behavioral, electrophysiological, and dopaminergic activity. These diverse effects are thought to be partly due to differential actions of amphetamine on striatal and mesolimbic brain regions. In addition, a differential, dose-dependent action of amphetamine via the D1 versus D2 dopamine receptors may account for some of these effects. We have investigated the acute effects of in vivo amphetamine treatment on the D1 dopamine-sensitive adenylate cyclase system, and report here that there are biphasic and regional alterations in adenylate cyclase activity which occur in response to different doses of amphetamine.

Adult, female, Holtzman rats were treated with 1.0, 2.5, 7.5 mg/kg amphetamine, or an equal volume of saline, I.P., 30 minutes prior to decapitation. Crude membrane fractions were prepared for adenylate cyclase assay from homogenates of striatal or mesolimbic (olfactory tubercle plus nucleus accumbens) dissections. Adenylate cyclase activity was measured in response to GTP and the D1 agonist, SKF38393.

Our data indicate that striatal membranes exhibit a small but significant up-regulation of D1 mediated cyclase activity after a low dose of amphetamine (1.0 mg/kg), whereas at higher doses (2.5 or 7.5 mg/kg), a down-regulation of activity is observed. These alterations are evidenced by a 12% increase in Vmax after 1.0 mg/kg amphetamine, compared to a 26% or 16% decrease in Vmax after 2.5 or 7.5 mg/kg, respectively, relative to controls. Mesolimbic brain regions, on the other hand, exhibit a down-regulation of D1-stimulated cyclase activity at all doses of amphetamine, with the maximum response (a 38% decrease in Vmax) occuring after 2.5 mg/kg amphetamine.

These results may be correlated with the multiphasic behavioral, electrophysiological, and biochemical effects of amphetamine that have previously been reported, suggesting a link between the regulation of adenylate cyclase activity and other responses which occur following acute amphetamine treatment.

Supported by NIMH grant 36044-04.

268.3 BEHAVIOURAL AND BIOCHEMICAL ALTERATIONS OF STRIATAL DOPAMINE RECEPTORS FOLLOWING REPEATED L-DOPA ADMINISTRATION IN 6-HYDROXY-DOPAMINE LESIONED RATS. A.Groppetti*, F.Tirone*, C.Flauto*, E.Paratì*, A.Vescovi* and M.Parenti. Dept. of Pharmacol., Univ. of Milan and Inst.Neurol. "C.Besta", Milano, Italy.

It has been reported that prolonged administration of L-dopa can cause behavioural changes reflecting enhanced activity of dopamine (DA) receptors. However, no biochemical correlation which might explain these behavioural alterations was found.

Now we report that L-dopa, given for two weeks to rat bearing a lesion of the nigro-striatal DA fibers, induced behavioural and biochemical changes both indicative of supersensitivity of DA receptors.

The lesions were performed by injecting 6-hydroxydopamine (6-OHDA) monolaterally into the rat substantia nigra. L-dopa was administered twice a day at a dose of 25 mg/kg (plus 2.5 mg/kg carbidopa) for two weeks starting 15 days after 6-OHDA injection. Sixteen hours after last L-dopa treatment rats were tested for apomorphine (0.5 mg/kg s.c.) induced circling behaviour or killed. Adenylate cyclase (AC) was assayed in partially purified striatal membranes. Striatal contents of Met-enkephalin-like immunoreactive material (ME-IR) and substance P (SP) were also measured.

It was found that, as expected, unilateral destruction of the nigro-striatal pathway with 6-OHDA induced controlateral rotations after apomorphine injection and increased sensitivity of AC to DA stimulation.

Treatment with L-dopa did not correct the increased sensitivity of AC to DA induced by 6-OHDA but further enhanced it. Also apomorphine-induced controlateral rotations were strongly potentiated by L-dopa. This effect was antagonized by SCH-23390, a compound reported to be a preferentially inhibitor of $D_1$, receptors. Striatal levels of both ME-IR and SP were altered by L-dopa treatment.

It is suggested that repeated administration of L-dopa to rats with nigro-striatal DA fibers lesioned, can enhance behaviour mediated by apomorphine and function of striatal DA receptors coupled to AC. It may be speculated that alterations of peptidergic modulatory influences are involved in these effects.

Supported by CNR and MPI Grants.

268.4 CHANGES IN RAT STRIATAL DOPAMINE-STIMULATED ADENYLATE CYCLASE ACTIVITY FOLLOWING ACUTE AMPHETAMINE ADMINISTRATION. J.V. Barnett* and R. Kuczenski. Tennessee Neuropsychiatric Institute and Dept. of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN 37232

Catecholamine receptors positively coupled to adenylate cyclase have been shown to undergo desensitization following excessive stimulation. Since amphetamine (AMPH) is known to act by releasing dopamine (DA) we chose to examine, for adaptive changes, striatal $D_1$ DA receptors which are positively coupled to adenylate cyclase. Doses of AMPH were chosen for their contrasting behavioral effects: 1 mg/kg which primarily enhances locomotor activity, and 5 mg/kg which promotes intense, focused stereotypies. AMPH or saline was administered subcutaneously 45 minutes prior to sacrifice. Following decapitation the striata were rapidly removed, homogenized and assayed for adenylate cyclase activity by following the conversion of $\alpha^{32}P$ ATP to $^{32}P$ cAMP. A dose of 5 mg/kg AMPH, but not 1 mg/kg AMPH, caused a 2 fold shift to the right in the dose response curve for DA in stimulating adenylate cyclase activity when compared to saline treated animals. Basal activity or guanosine 5'-triphosphate (10-5 M), guanosine 5'-(β-γ-imido)triphosphate (10-5 M), and NaF (10 mM)-stimulated activity did not change. The time course of the apparent AMPH-mediated $D_1$ DA receptor densensitization was next addressed. Groups of animals (n=6) were administered 5 mg/kg AMPH and sacrificed at 0, 25, 90, and 180 minutes post administration. Desensitization was noted at 25 minutes while cyclase activity at 90 and 180 minutes did not differ significantly from the 0 time point. At no time point tested (25, 45, or 60 minutes) did 1 mg/kg AMPH alter DA-stimulated adenylate cyclase activity. Desensitization of the DA-stimulated adenylate cyclase was also observed at a stereotypy producing dose of methylphenidate (MEPH) (50 mg/kg at 40 minutes). These data demonstrate $D_1$ DA receptor desensitization. The lack of change in NaF-stimulated activity is consistent with homologous desensitization as described for other cyclase systems. This desensitization occurs following a stereotypy-producing dose of both AMPH and MEPH. A possible role for $D_1$ DA receptor densensitization in the onset of stereotypy is suggested. (This work is supported by USPHS Grant DA 02676 and J.V.B. is supported by NIH Training Grant GM-07628).

268.5 **EFFECTS OF AN IRREVERSIBLE DOPAMINERGIC LIGAND, FLUPHENAZINE-N-MUSTARD (FNM), ON DOPAMINERGIC BEHAVIOR IN SUPERSENSITIVE MICE.** K. Thermos, J.D. Winkler*, D.B. Goodale* and B. Weiss, Department of Pharmacology, Medical College of Pennsylvania, 3300 Henry Avenue, Philadelphia, PA 19129

FNM irreversibly inhibits [$^3$H]spiroperidol binding to mouse striatal membranes both in vitro and in vivo. In vitro FNM inhibits [$^3$H]spiroperidol binding with an $IC_{50}$ of 80 nM. Preincubating FNM (250 nM) with striatal membranes, followed by extensive washing, reduced the $B_{max}$ of [$^3$H]spiroperidol by approximately 60%, indicating the irreversible nature of this ligand. In vivo administration of FNM ip to mice reduced [$^3$H]spiroperidol binding in a dose-dependent manner with an $ED_{50}$ of 2.5 umole/kg, as determined by the binding of a single concentration of spiroperidol (0.5 nM). To elucidate whether FNM binds irreversibly to [$^3$H]spiroperidol binding sites in vivo, we administered a single dose of 62 umole/kg, ip, to mice. The striatal tissue was removed two hours after injection and extensively washed. Scatchard analysis of [$^3$H]spiroperidol binding (0.1-4 nM) revealed a 50% decrease in $B_{max}$. This agrees with the in vitro evidence for the irreversibility of FNM binding. In order to determine the effects of FNM on dopaminergic function, we examined the ability of FNM administered in vivo to antagonize apomorphine-induced rotational behavior. We used mice with unilateral lesions of the striatum caused by injection of 6-hydroxydopamine using a plastic mold described by Goodale et al., (Soc. Neurosci., 1985). In these animals, acute apomorphine injection caused a consistent rotational behavior, with an $ED_{50}$ of 0.4 umole/kg, sc. FNM was able to block the rotational behavior induced by an injection of apomorphine (1 umole/kg,sc) in a dose related fashion. The $ED_{50}$ for FNM was approximately 0.8 umole/kg, ip; complete blockade occurred at 20 umole/kg, ip. A high dose of FNM (100 umole/kg, ip) completely blocked the apomorphine-induced rotation and reduced the $B_{max}$ of the lesioned striatum by 85%. Administration of a low dose of FNM (1.8 umole/kg, ip), however, caused a 90% reduction in apomorphine-induced rotation with no significant effect on the $B_{max}$ of [$^3$H]spiroperidol binding. This preliminary evidence indicates that the behavioral effects of FNM can be observed at doses that do not appear to alter [$^3$H]spiroperidol binding, suggesting that the blockade of certain dopaminergic behavioral effects by FNM may be explained by its interaction with sites besides spiroperidol binding sites. Accordingly, this irreversibly binding dopaminergic ligand may be a useful probe for identifying proteins involved in dopamine mediated behaviors. Supported by funds from the Department of Public Welfare, Commonwealth of Pennsylvania.

268.6 DECREASED DOPAMINERGIC D-2 BINDING IN THE GLOBUS PALLIDUS OF HYPERPROLACTINEMIC RATS. N.S. PILOTTE, K.A. GREGERSON and D.R. BURT. Depts. Pharmacol. and Physiol., Univ. of Maryland Sch. of Medicine, Baltimore, MD 21201.

Others have reported that striatal dopaminergic D-2 binding sites, evaluated by incubation of membranes with [$^3$H]spiperone in the presence or absence of d(+)butaclamol, slightly increase or do not change in rats made hyperprolactinemic by chronically elevated levels of estradiol or prolactin (PRL). However, spiperone (with butaclamol) occupies serotoninergic S-2 sites in the striatum as well and the presence of these additional sites may obscure differences in binding to D-2 receptors.

Cycling female rats were made hyperprolactinemic by treatment with ovine PRL (oPRL, 4 mg/kg sc, q 8 hr). Treatment was initiated on the morning of diestrus 2 and and rats were decapitated 49 hr after the first injection. Each brain was transected coronally at the anterior aspect of the optic chiasm and the globus pallidus and caudate nucleus were removed separately and homogenized. The washed resuspended membranes were incubated at 37°C with 0.4 nM [$^3$H]spiperone in the presence of absence of $10^{-6}$M d(+)butaclamol or $10^{-7}$M quinazolinedione (R43448) for 30 min. Bound ligand was separated from free by vacuum filtration through Whatman GF/B filters and washed with 12 ml ice-cold 0.15 M NaCl. The number of sites bound in the presence of R43448 was subtracted from those bound in the presence of butaclamol and represented the number of D-2 sites. Two-thirds of the sites labeled by [$^3$H]spiperone in the caudate were dopaminergic and their numbers were similar in rats treated with vehicle or oPRL (273 $\pm$ 42 and 316 $\pm$ 28 fmol/mg protein, respectively). About 50% of the sites labeled by [$^3$H]spiperone in the globus pallidus were dopaminergic in each group. However, treatment with oPRL resulted in a 50% reduction in D-2 sites in the globus pallidus compared to vehicle-treated controls (69 $\pm$ 10 and 132 $\pm$ 39 fmol/mg protein, respectively). These results are suggestive that the dopaminergic sites in the globus pallidus, which comprises about 25% of the striatum, may be hormonally sensitive whereas the remaining caudate is not.

268.7 REGULATION OF STRIATAL D2 DOPAMINE RECEPTORS. H. R. Wagner, M. Traub*, A. Reches, G. Nilaver, E. Alter*, V. Lewis, S. Fahn, E.A. Zimmerman. Depts. of Pharmacology and Neurology, Columbia Univ. Coll. of P. & S., NY, NY 10032.

Dopamine (DA) D2 receptors in rat brain up-regulate following interruption of dopaminergic function. Up-regulation is induced by denervation, transmitter depletion or receptor blockade. The mechanism underlying D2 up-regulation has been assumed to be the same regardless of the method of induction. However recent studies demonstrating haloperidol (HAL)-induced increases in the density of $^3$H-SPIP binding sites in the denervated mesolimbic area (Staunton et al., Nat.,299:72,1982) and striatum (Reches et al., Br. Res.,275:183,1983) challenge this assumption and suggest that denervation and receptor blockade affect D2 receptors through separate mechanisms. We now report that chronic administration (two weeks) of the DA depleting agent, reserpine(RES)(0.25 mg/kg, sc), to rats with unilateral 6-OHDA lesions of the substantia nigra also induces an additive increase in the density of $^3$H-SPIP striatal binding sites. Increases occur at doses of RES producing maximal increases in the density of $^3$H-SPIP binding sites in intact striata. Denervation-induced up-regulation of D2 receptors differs from drug-induced up-regulation. Additivity does not occur in intact rats treated concurrently with RES and HAL (2.0 mg/kg, ip) or with RES and the tyrosine hydroxylase inhibitor, alpha-methylparatyrosine (a-MPT) (75.0 mg/kg, ip). Additive increases in binding in denervated animals receiving RES are not mediated by a RES-induced decrease in striatal DA levels. Doses of RES above those used in our studies (0.25 mg/kg, sc) produce further decreases in striatal DA levels but no increase in the density of $^3$H-SPIP binding sites. Concurrent administration of a-MPT and RES also decreases striatal DA below levels obtained in rats receiving only RES but is without effect on $^3$H-SPIP binding. The latter findings confirm that additivity is not mediated by drug-induced decreases in the amount of residual DA. The data also show that denervation-induced up-regulation of striatal D2 binding sites differs from drug-induced up-regulation. We speculate that the unique effect of denervation on the D2 receptor may reflect the loss of a presynaptic regulatory factor.

268.8 ALTERATIONS IN THE AFFINITY OF [$^3$H]NIMODIPINE BINDING SITES IN THE BRAIN FOLLOWING CHLORPROMAZINE TREATMENT AND SUBSEQUENT WITHDRAWAL. V. Ramkumar* and E.E. El-Fakahany (SPON: G.A. Young). Dept. of Pharmacology and Toxicology, Univ. of Maryland School of Pharmacy, Baltimore, MD 21201.

The following study was undertaken to assess possible alterations at the calcium channel level in the brain following chronic chlorpromazine treatment and subsequent drug withdrawal. Male ICR mice were administered chlorpromazine orally in ground diet mixed with the drug (0.5 mg chlorpromazine/g diet. The estimated drug intake per animal with this dose was 2.5 mg/day. [$^3$H]nimodipine binding studies were performed 1 and 2 months following initiation of drug treatment and 1, 2, and 3 weeks following subsequent withdrawal after a 2 month treatment. For binding studies, brains were homogenized in 40 vol. of 50 mM Tris-Hcl buffer (pH 7.4) and crude synaptosomal membranes were prepared by differential centrifugation. The final pellet was resuspended in buffer to give a 5% homogenate (w/v). Membranes were incubated with 5-8 concentrations of [$^3$H]nimodipine (130.5 Ci/mmol, New England Nuclear, Boston, MA) in a final volume of 1 ml of Tris buffer, with 1 uM nifedipine used to define non-specific binding. Incubations were for 90 min in the dark at room temperature. Samples were then filtered under vacuum through GF/C glass fiber filters and washed 3 times with 3 ml ice-cold Tris buffer. Radioactivity was determined by liquid scintillation spectrophotometry.

Our results indicate significant increases in the affinity constant ($K_a$) of [$^3$H]nimodipine-$Ca^{++}$ channel complex with chlorpromazine treatment compared to age-matched controls. These values averaged 6.35, 10.64 and 15.87 ($nM^{-1}$) for control mice and for those treated with chlorpromazine for 1 and 2 months, respectively. Maximal binding capacity ($B_{max}$) did not show significant changes from control, averaging 185.8, 188.7 and 155.4 (fmol/mg protein) for the respective groups. Upon withdrawal of chlorpromazine a significant decrease in the $K_a$ was observed, without significant alteration in $B_{max}$. Values for $K_a$ averaged 6.67, 1.98 and 7.8 ($nM^{-1}$) following 1, 2 and 3 weeks of withdrawal, respectively, with the corresponding $B_{max}$ values of 182.4, 203.0 and 173.0 (fmol/mg protein). The implications of these findings in terms of the therapeutic and side effects of this neuroleptic drug are not clear at present. Studies presently underway, assessing the effects of chronic treatment with other neuroleptic drugs on [$^3$H]nimodipine binding, may contribute to a better understanding of these preliminary findings. (Supported in part by a grant from Miles Laboratories)

268.9 A ROLE FOR PRESYNAPTIC BLOCKADE IN THE DEVELOPMENT OF A NEUROLETIC INDUCED DOPAMINE RECEPTOR HYPERSENSITIVITY. J.K. Clopton*, W.C. Koller, J.C. Curtin and J.H. Gordon. Dept. of Pharmacology, The Chicago Medical School, N. Chicago, IL 60064 and Hines V.A. Medical Center, Chicago, IL.

Existing data suggest that two benzamide compounds metaclopromide (MET) and sulpiride (SUL) are about equally potent in terms of their ability to block postsynaptic dopamine receptors (i.e. ability to inhibit apomorphine-induced turning behavior or stereotypy). However, MET is 20 times more potent than SUL in blocking dopamine autoreceptor mediated events (i.e. apomorphine-induced hypomotility). Because of this divergence in the relative pre vs postsynaptic blocking potency , it was possible to conduct an experiment to determine what role, if any, presynaptic dopamine autoreceptors would play in the development of a neuroleptic-induced postsynaptic dopamine receptor hypersensitivity.

Male Sprague-Dawley rats received SUL (10 mg/kg/day; N=6), MET (10 mg/kg/day; N=6) or a ratio of the two (SUL/MET= 9/1 or 7/3 mg/kg/day; N=6/group) for 16 days. These experimental groups essentially hold the relative amount of postsynaptic blockade constant while varying the relative amount of presynaptic blockade. Additional groups of animals received 1 or 3 mg/kg/day of MET or 20 mg/kg/day of SUL. All experimental and control groups were tested for apomorphine-induced stereotyped behavior 6 days after the last dose of neuroleptic.

MET at a dose of 10 mg/kg/day induced a postsynaptic dopamine receptor hypersensitivity, while the same dose of SUL had no effect. However, at equal mg doses, MET is 20 times more potent than SUL at blocking dopamine autoreceptors. The results of the relative "presynaptic dopamine receptor blockade" dose response curve illustrated that increasing the level of chronic presynaptic dopamine receptor blockade results in an increased postsynaptic dopamine receptor sensitivity upon withdrawl. Since the level of postsynaptic receptor blockade was held constant it would appear that some level or proportion of presynaptic dopamine autoreceptor blockade is required before the chronic postsynaptic blockade will result in the development of a postsynaptic dopamine receptor hypersensitivity. Interestingly, it is probably the combination of pre- and postsynaptic blockade which produces this response since 3 mg/kg/day of MET alone is not effective but this dose is effective when administered with 7 mg/kg/day of SUL. Overall our preliminary results and data from the literature suggest that a blockade of the dopamine autoreceptors is required before chronic blockade of postsynaptic dopamine receptors will result in the development of a receptor hypersensitivity. Moreover, these data suggest that the presynaptic dopamine autoreceptor may be responsible for the development and maintainance of a neuroleptic-induced dopamine receptor hypersensitivity.

268.10 QUANTITATIVE AUTORADIOGRAPHIC ANALYSIS OF INCREASED [$^3$H]-SPIROPERIDOL BINDING FOLLOWING CHRONIC HALOPERIDOL TREATMENT. C.M. Paden, S. Krueger* and T.C. Rainbow[1]. Dept. of Biology, Montana State Univ., Bozeman, MT 59717 and Dept. of Pharmacology, Univ. of Pennsylvania School of Medicine, Philadelphia, PA 19104.

While the ability of chronic neuroleptic treatment to elevate dopamine (D-2) receptors in the neostriatum has been known for almost a decade (D.R. Burt et al., Science 196: 326, 1977), the limitations of membrane homogenate binding methods have made it impossible to examine this phenomenon in more anatomically discrete brain areas. New techniques in computer-assisted densitometry of film autoradiograms have recently been applied to analysis of increases in neostriatal D-2 receptors following lesions of the substantia nigra (K.A. Neve et al., Brain Res. 302: 9, 1984), indicating the potential of quantitative autoradiography for anatomically precise investigation of receptor plasticity. In order to demonstrate the applicability of this approach to drug induced alterations in D-2 binding, we have performed densitometric equilibrium binding analyses on serial sections of neostriatum from control and neuroleptic treated rats.

Adult male Holtzman rats received either vehicle, 0.5 mg/kg or 10 mg/kg haloperidol sc daily for 3 weeks. Neuroleptic treated animals displayed marked catelepsy shortly after injections. Animals were sacrificed by decapitation 6 days after cessation of drug treatment, and brains were removed and frozen on dry ice for Ultrofilm autoradiography as previously described (T.C. Rainbow et al., J. Neurosci. Methods 5: 127, 1982). Three sets of 12 serial sections were cut at different rostral-caudal levels of the neostriatum. One section of each set was incubated in one of 9 concentrations of $^3$H-spiroperidol (21 Ci/mmole, NEN) ranging from 0.064 to 1.49 nM or in one of 3 concentrations of ligand plus 1 μM + butaclamol. Incubation conditions were as described by J.M. Palacios et al., Brain Res. 213: 277, 1981. Following incubation sections were rinsed, dried and apposed to LKB Ultrofilm (T.C. Rainbow et al., ibid) for 60 days.

Initial Scatchard analyses of the most caudal series of autoradiograms using a computerized video image analysis system (Eye Com II; Spatial Data Systems) revealed that increases in saturable binding of $^3$H-spiroperidol are readily quantifiable on film autoradiograms. Maximum binding in whole striatum was increased 37% and 45% over that of controls in the low and high dose haloperidol groups, respectively. These preliminary findings indicate that computerized densitometry of film autoradioagrams should be useful in quantifying drug induced alterations in dopamine receptor binding in more restricted anatomical areas too small for analysis in homogenate binding assays.

[1]Deceased.

268.11 PROLACTIN ENHANCES THE RATE OF TIME-DEPENDENT DECLINE IN CATELEPSY INDUCED BY IRREVERSIBLE NEOSTRIATAL DOPAMINE RECEPTOR BLOCKADE VIA N-ETHOXYCARBONYL-2-ETHOXY-1,2-DIHYDROQUINOLINE (EEDQ). J.A. Joseph, J. Coupet, *+L. Antonian, C.C. Loullis. Dept. of CNS, Med.Res., Lederle Labs, Pearl River, NY and +Matrix Labs, New York, NY.

Previous experiments have indicated that rats given EEDQ, a potent, irreversible D-2 receptor antagonist), exhibit catalepsy and other behavioral effects indicative of D-2 receptor blockade. In vitro receptor binding assays of striatal membranes from these animals have revealed declines in Bmax of > than 90%. These effects are time-dependent and usually disappear within one week following injection. In the present experiment, attempts were made to determine whether this time course could be altered by prolactin administration. Prolactin has been shown to induce striatal dopamine receptor up-regulation. Thus, the effects of chronic prolactin were examined on the rates of (a) reappearnace of neostriatal D-2 receptors and (b) the declines in catalepsy during the first 48 hours after EEDQ (10 mg/kg) treatment in Wistar rats. Two groups of rats were utilized. They received subcutaneous implants of Alza pumps containing either rat prolactin dissolved in saline (35.2 μg in 220 μl; infusion rate 160 ng/hr) or saline. EEDQ (10 mg/kg i.p.) was administered on day 6 following surgery. Catalepsy was assessed at 4 hrs, 24 hrs and 48 hrs after EEDQ administration by observing the length of time that the animals remained stationary with their forpaws on a horizontal rod and their hindpaws on the floor. Subgroups of saline or prolactin treated animals were sacrificed at the above timepoints and [$^3$H]spiroperidol binding determined in striatal membrane preparations from these animals and non EEDQ injected controls. Results showed that while the prolactin-treated animals exhibited greater catalepsy than saline treated animals at 4 hrs post EEDQ (X Pro 131.2 ± 10.2 sec; N = 11; SAL X 80.9 ± 23.0 sec N = 11). The prolactin treated animals showed greater recovery than saline treated animals (X Pro 11.8 ± 2.2; X sal 23.2 ± 13.9) at 24 hrs following EEDQ treatment. These findings contributed to a significant condition x time interaction in a subsequent analysis of variance ($F(1,20) = 6.1 / < .025$). At 48 hrs post EEDQ these differences were no longer apparent because all animals had recovered. Findings from the preliminary $^3$H spiroperidol binding analyses revealed that the reappearance of $D_2$ receptor binding at these time points paralleled the behavioral recovery (Sal and Pro at 4 hrs $^3$H spiroperidol binding = 0% of control; at 24 hrs Pro = 41% Sal = 20% of control; at 48 hrs 50% and 39% respectively).

268.12 CALCIUM CHANNEL AGONIST-INDUCED MURINE SEIZURES. R.C. Shelton*, J.A. Grebb and W.J. Freed. (SPON: G.N. Ko). Section on Preclinical Neurosciences, Neuropsychiatry Branch, NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032.

Calcium has been implicated in both the pathophysiology of seizures and the mechanism of action of some anticonvulsants. Calcium channel inhibitors (CCI's) and agonists are potential pharmacologic probes of these relationships. We report here that the calcium channel agonist Bay K 8644 induces seizures in mice. We also report the effects of pretreatment with various CCI's and anticonvulsants on Bay K 8644-induced seizures.

**METHODS:** A total of 70 Swiss-Webster mice were used. Bay K 8644 was injected freehand intraventricularly (ICV) into restrained animals (J. Appl. Physiol., 25:319, 1968). Animals were observed for 60 min and seizures were scored for time of onset, duration and severity. Dose response experiments determined that 37.5 μg ICV was the minimum dose producing consistent seizure activity (duration = 19.3 ± 6.4 min (mean ± SEM)). The effects of CCI's and anticonvulsants on seizure activity were assessed by pretreating the animals with intraperitoneal injections of the test drugs 30 min before administering the Bay K 8644.

**RESULTS:** Bay K 8644 produces seizures in mice characterized by recurrent clonic and tonic convulsions sometimes accompanied by rigidity and self mutilation. Four different CCI's were tested for their ability to block Bay K 8644-induced seizures. Nifedipine (50 mg/kg) and verapamil (50 mg/kg) completely blocked the seizures. Diltiazem (50 mg/kg) and nifedipine (5 mg/kg) reduced the duration to 8.0 ± 3.6 min and 9.7 ± 3.7 min, respectively. These drugs also reduced the severity of the seizures. Flunarizine (50 mg/kg) did not affect the seizures. Five conventional anticonvulsants were also tested. Phenytoin (50 mg/kg) and valproate (200 mg/kg) increased the duration of seizures to 54.5 ± .69 min and 50.7 ± 2.0 min, respectively, and also increased the severity of the seizures. Carbamazepine (50 mg/kg), diazepam (5 mg/kg) and pentobarbitol (32.5 mg/kg) did not affect the seizures.

**DISCUSSION:** The most important finding is that Bay K 8644, thought to be a fairly specific calcium channel agonist, induces seizures in mice which lends additional support to studies suggesting a role of brain calcium regulation in seizures. The ability of nifedipine and verapamil to block these seizures supports their being induced by calcium channel agonist activity. CCI's previously have been reported to block metrazol-, 4-aminopyridine-, penicillin-, lesion-, and audiogenic-induced seizures in other murine models. The exacerbation of Bay K 8644-induced seizures by phenytoin is perhaps surprising in view of the report that phenytoin inhibits the binding of nitrendipine to brain memebranes (Ann. Neurol., 16:616, 1984). Further investigations with this model, however, are encouraged by a study showing beneficial clinical results in children with temporal lobe epilepsy when treated with flunarizine (Epilepsia, 25:217, 1984).

268.13 **CALCIUM CHANNEL INHIBITORS PREVENT NEUROLEPTIC-INDUCED APOMORPHINE SUPERSENSITIVITY IN MICE.** J.A. Grebb, R.C. Shelton*, and W.J. Freed. Section on Preclinical Neurosciences, Neuropsychiatry Branch, NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032.

Chronic thioridazine (TDZ) treatment in animals produces less dopaminergic supersensitivity than other neuroleptics. We hypothesized that this difference is due to the potent calcium channel inhibitory effect of TDZ (Am. J. Psychiatry, 141:352, 1984). If this hypothesis were correct, the effects of TDZ would be mimicked by coadministration of calcium channel inhibitory (CCI) drugs with haloperidol (HAL), a neuroleptic without CCI activity.

**METHODS:** Swiss-Webster mice (n=163) were treated chronically (28d) with CCI's (diltiazem, nifedipine or verapamil) in two different doses (high and low) and/or HAL. Powdered CCI was mixed with powdered food, and HAL was administered in the drinking water. Following three days of drug withdrawal, mice were tested for amphetamine (AMPH)-induced locomotion and apomorphine (APO)-induced cage climbing. Data were analyzed by a one-way analysis of variance followed by a Duncan's multiple comparisons test. The null hypothesis was rejected at the .05 level.

**RESULTS:** HAL treatment alone resulted in an APO-induced cage climbing score of 46.3 ± 3.0 (mean ± SEM), and animals that received neither CCI or HAL had a score of 31.5 ± 3.2. Coadministration of high-dose diltiazem or verapamil (but not nifedipine) prevented the development of HAL-induced APO supersensitivity with scores of 22.5 ± 8.8 and 33.6 ± 7.1, respectively, with low-dose diltiazem and verapamil showing a trend in the same direction. Chronic administration of diltiazem without HAL also induced APO supersensitivity (54.0 ± 2.8). Coadministration of CCI's with HAL did not affect the development of HAL-induced AMPH supersensitivity; however, verapamil treatment alone induced AMPH supersensitivity.

**DISCUSSION:** The data support the hypothesis that coadministration of a CCI (verapamil or diltiazem, but not nifedipine) with HAL would mimick the effects of TDZ treatment alone. The differential effects among the CCI's are consistant with the finding that TDZ has diltiazem-like effects on dihydropyridine (e.g., nifedipine) binding on rat myocardial membranes (Biochem. Biophys. Res. Commun., 121:657, 1984), as well as with the hypothesis that diltiazem and verapamil share a common binding site (Proc. Natl. Acad. Sci. U.S.A., 80:860, 1983). The different results for APO and AMPH sensitivity could be explained by hypothesizing that CCI's blocked the spread of dopamine receptors to extra-synaptic regions of the neuronal membrane. The reasons for the diltiazem-induced APO supersensitivity and the verapamil-induced AMPH supersensitivity are unknown, but suggest complex and potentially important interactions between calcium channels and dopaminergic systems.

268.14 SEROTONIN TYPE 2 (S2) RECEPTOR BINDING IN MONKEY AND RAT BRAINS FOLLOWING BASAL FOREBRAIN LESIONS WITH IBOTENIC ACID. G.Wenk,K. Engisch*,L.McCall*T.Aigner,S.Mitchell,R.Struble,D.Price,M.Mishkin and D.Olton. Departments of Psychology and Neuropathology, The Johns Hopkins University, Baltimore, MD 21218 and Laboratory of Neuropsychology, NIMH, Bethesda MD 20205.

In monkeys and rats, the major source of cholinergic (CH) innervation of the neocortex and hippocampus is the nucleus basalis magnocellularis (NBM) and medial septal area (MSA). The death of CH neurons in the NBM and MSA causes reductions in cortical CH markers which correlate with the presence of dementia in humans with Alzheimer's Disease (AD) and with impaired memory in rats with basal forebrain lesions. Serotonin neurons originating in the dorsal and median raphe nuclei modulate NBM CH influence by tonically inhibiting the release of acetylcholine in the cortex and hippocampus. AD patients show a significant decrease of serotonergic receptors, serotonin and 5-HIAA levels in the cortex and hippocampus. We investigated whether the loss of CH afferents to the cortex and hippocampus in monkeys and rats correlates with changes in serotonergic innervation.

Nine male cynomologus macaques (Macaca fascicularis),3-5 kg, were divided into experimental (lesion) and control groups. Lesions of NBM and MSA were made with ibotenic acid. 10 months after surgery, the monkeys were sacrificed and bilateral samples were taken from the hippocampus and 28 cortical areas and assayed for choline acetyltransferase (ChAT) activity and S2 binding. 60 male albino rats (250-300g) were divided into experimental (NBM and MSA lesions) and control groups and sacrificed 4 months after surgery. Rat cortex and hippocampus were assayed for ChAT activity, S2 and QNB receptor binding, high affinity choline uptake (HACU) and endogenous levels of biogenic amines.

In monkeys, cortical ChAT activity decreased and S2 binding increased and were inversely correlated. The hippocampus was an exception to this pattern; ChAT activity decreased and S2 binding did not change. In rats, frontal cortical ChAT activity decreased and S2 receptor sites increased. Hippocampal ChAT decreased and S2 binding did not change. Cortical and hippocampal QNB receptor binding and norepinephrine, dopamine, and serotonin levels were not different from controls. HACU was decreased in both areas. The results suggest that non-cholinergic neurotransmitter systems may respond to the loss of cortical cholinergic afferents. Supported by USAMRDC DAMD-17-C2225, AG05146 and NS20471.

268.15 QUANTITATIVE AUTORADIOGRAPHY OF CHRONIC ANTIDEPRESSANT AND ESTROGEN TREATMENT ON SEROTONIN-2 RECEPTORS IN RAT BRAIN. C. Fischette, Dept. of Pharmacology, Hoffmann-La Roche Inc., Nutley, NJ 07110

Numerous studies using membrane fractions or homogenates of rat brain have indicated that chronic administration of all classes of antidepressant drugs lowers serotonin-2 receptors (S-2) in the forebrain. Estrogen has also been used as an effective antidepressant, and studies in the literature indicate that antidepressant-induced down-regulation of S-2 receptors does not occur in gonadectomized male or female rats unless estrogen is present. While studies using grossly dissected brain regions are valuable, the quantitative autoradiographic technique allows examination of discrete areas of the brain that were previously unavailable for study. Hence, individual nuclei and discrete layers of the cortex can be analyzed.

All groups of male and female rats were gonadectomized for 7 days prior to chronic imipramine treatment. At the time of gonadectomy, subgroups of males and females were implanted subcutaneously with silastic capsules containing 100% crystalline estradiol (1 cm exposed steroid). Imipramine or vehicle was injected i.p. once daily (10 mg/kg/day) for 21 days. Twenty-four hours after the last injection all animals were sacrificed and the brains were prepared for autoradiography of S-2 receptors. Briefly, 32µ sections of rat brain were dried and labelled with 2.0 nM $^3$H-Ketanserin (67 Ci/mmol) ± 1 µM methysergide to assess nonspecific binding. Sections were apposed to LKB Ultrofilm along with tritiated brain mash "standards". Various areas of the CNS were analyzed by computer-assisted densitometry.

As previously described, an intense layering of $^3$H-Ketanserin binding is found in frontal cortex (Fischette, C.T. and Nock, B., Soc. for Neurosci., p. 554, 1984). Middle layers of cortex bind to a great extent while outer and inner layers of cortex are only moderately labelled. Analysis of the frontal cortex indicates that down-regulation of S-2 receptors in response to estrogen + imipramine occurs in all layers, even though receptors are not homogeneously distributed in this area. Data from other relevant limbic nuclei will be presented. Specific localization of action may give us insights into the mechanism of action of antidepressant drugs.

268.16 ANTIDEPRESSANT AGENTS ALTER THE SENSITIVITY OF 5-HT RECEPTORS INVOLVED IN THERMOREGULATION. G.A. Gudelsky, J.I. Koenig, H. Jackman and H.Y. Meltzer, Univ. of Chicago, Dept. of Psychiatry and Ill. State Psychiatric Institute, Chicago, IL.

Treatment of rats with tricyclic antidepressants has been shown to reduce the number of 5-$HT_2$ binding sites without influencing the number of 5-$HT_1$ sites. On the other hand, monoamine oxidase inhibitors (MAOI) have been shown to reduce the number of both 5-$HT_2$ and 5-$HT_1$ receptors. Recently we have proposed that in the rat 5-$HT_2$ receptors mediate 5-HT agonist-induced hyperthermia while 5-$HT_1$ receptors mediate 5-HT agonist-induced hypothermia (Pharm. Biochem. Beh. 22:489, 1985). The present study was undertaken in order to determine whether the responsiveness of 5-HT receptor mechanisms involved in thermoregulation is altered after the administration of antidepressant agents. The hyperthermic response resulting from the activation of 5-$HT_2$ receptors by 6-chloro-2-(1-piperazinyl)-pyrazine (MK-212) was examined in rats 48 hrs after the administration of mianserin (10 mg/kg). Mianserin treatment produced a significant shift to the right in the dose-response relationship for MK-212-induced hyperthermia. The mianserin-induced suppression of the hyperthermic response to MK-212 was dose-related and observed 48 hrs but not 24 or 96 hrs after its administration. Mianserin treatment also diminished the increase in body temperature elicited by 5-methoxy-N,N-dimethyltryptamine (5MeODMT, 3 mg/kg). The hyperthermic response to MK-212 also was diminished 48 hrs after the administration of several other agents known to reduce the number of 5-$HT_2$ receptors, viz., pizotifen (5 mg/kg), loxapine (10 mg/kg) and methysergide (10 mg/kg). In contrast, the 5-$HT_{1A}$ receptor-mediated decrease in body temperature produced by 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) was not altered by mianserin. Treatment of rats with the MAOI nialamide (75 mg/kg), twice daily) for 7 days significantly diminished the 5-$HT_2$ receptor-mediated increase in body temperature produced by 5MeODMT, as well as the 5-$HT_{1A}$ receptor-mediated decrease in body temperature produced by 8-OH-DPAT. The effect of chronic nialamide on MK-212-induced hyperthermia could not be studied because a single injection of nialamide attenuated the response to MK-212. It is concluded that the sensitivity of 5-$HT_2$ and 5-$HT_{1A}$ receptors involved in thermoregulation can be altered by antidepressant drugs in a manner that is consistent with data from radioligand binding studies. Thus, the administration of mianserin, loxapine, pizotifen or methysergide selectively desensitizes 5-$HT_2$ receptor mechanisms involved in hyperthermic responses, whereas nialamide reduces the responsiveness of both these receptors and 5-$HT_{1A}$ receptors involved in hypothermic responses.

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268.17 COMPARISON OF SEROTONIN AGONIST AND ANTAGONIST EFFECTS ON 5-$HT_2$ BINDING SITES. M. A. Blackshear, L. L. Martin* and E. Sanders-Bush. Department of Pharmacology, Meharry Medical College, Nashville, TN; Neuroscience Res., CIBA-GEIGY Corp., Summit, NJ; and Department of Pharmacology, Vanderbilt University, School of Medicine, Nashville, TN 37208.

Previous studies have shown that acute and chronic administration of the serotonin (5-HT) antagonist, mianserin, decreases $^3$H-spiperone binding to 5-$HT_2$ sites in the frontal cortex (Arch. Pharmacol. 324, 125, 1983). Using $^3$H-ketanserin, a more specific ligand for the 5-$HT_2$ site, the present study confirms that acute mianserin causes a decrease in 5-$HT_2$ sites and further investigates the mechanism of this effect. The in vitro and in vivo effects of mianserin are compared with those of trifluoromethylphenylpiperazine (TFPP), a directly acting 5-HT agonist.

Male Sprague-Dawley rats were administered single doses of 5 mg/kg mianserin or single or repeated doses of 20 mg/kg TFPP. One day after the last injection, 5-$HT_2$ binding sites in the frontal cortex were assayed. Chronic administration of TFPP twice daily for 5 days caused a 48% decrease in the density of 5-$HT_2$ sites (Bmax = 25.7 ± 1.1 vs 13.3 ± 0.6 pmol/g for control and treated rats), with no change in $k_d$ (0.69 ± 0.04 vs 0.79 ± 0.07 nM). The adaptive change in 5-$HT_2$ sites induced by chronic TFPP treatment was similar, both qualitatively and quantitatively, to that caused by chronic mianserin treatment. However, unlike mianserin, which also decreased 5-$HT_2$ sites after a single dose, the effect of TFPP was only evident when given chronically. Mianserin and TFPP had similar in vitro effects on 5-$HT_2$ sites. Preincubation of brain membranes with either 12 nM mianserin or 0.5 uM TFPP induced an increase in the $K_d$ value with no change in the Bmax value.

Similar adaptive changes in 5-$HT_2$ sites after treatment with mianserin, which presumably binds to this site as an antagonist, and after TFPP, which is thought to bind as agonist, is not the predicted response based upon other aminergic systems. It is possible, therefore, that adaptive mechanisms in the serotonergic system differ from those in other aminergic systems or, alternatively, that the drugs used to characterize 5-HT receptor systems should be re-evaluated. (Supported by a NSF fellowship to MAB, NIH Research Grants MH-26463 and MH-34007).

268.18 METHYSERGIDE TREATMENT SELECTIVELY DECREASES SEROTONIN-2 RECEPTORS IN MOUSE CEREBRAL CORTEX. P.C. May, D.G. Morgan, D. Salo* and C.E. Finch. Andrus Gerontology Ctr., USC, Los Angeles, CA 90089.

Serotonin receptor regulation is poorly characterized when compared to the well described supersensitization of dopaminergic and beta-adrenergic receptors in response to chronic receptor blockade or denervation. To assess serotonergic receptor plasticity, young (3 mo) male C57BL/6J mice were treated with methysergide, a serotonin antagonist with central actions at both serotonin-1 (S-1) and serotonin-2 (S-2) receptors. Methysergide (0, 5, 15, 45 mg/kg body weight; n=16/dose) was administered in the drinking water for 21 days. After a 3 day drug free period, mice were sacrificed and cerebral cortex (CX), hippocampus (HC), hypothalamus (HY) and caudate nucleus (CN) were collected and frozen. S-1 binding sites were measured in HC, HY and CX by saturation analysis using 6 concentrations of ($^3$H)5-HT (0.5-16 nM) with non-specific binding estimated with 10 uM methysergide. Assays were conducted under conditions which prevented membrane peroxidation or formation of a low affinity binding site for ($^3$H)5-HT. In addition, S-2 binding sites were measured simultaneously in CX membranes using ($^3$H)-spiperone (0.25-8 nM) with 30 nM prazosin added to all tubes to occlude the alpha-adrenergic site. Ketanserin (500 nM) was used to define non-specific binding. In control animals, S-1 receptor density determined by Scatchard analysis averaged 257 ± 17, 179 ± 4, 120 ± 7 fmoles/mg protein for HC, CX and HY, respectively. Methysergide treatment had no effect on S-1 receptor density or Kd (1.2 ± 0.1 nM). In contrast, methysergide treatment unexpectedly reduced S-2 binding in the CX in a dose-dependent fashion (8%, 21% and 28% decrease for 5, 15, 45 mg/kg methysergide, respectively). The effect was specific for serotonergic S-2 sites since $^3$H-spiperone binding in the CN, which labels primarily D-2 dopamine sites, was unaffected by drug treatment. These results suggest that serotonin receptors behave in a non-classical manner in response to chronic receptor blockade with antagonist or that in vivo, methysergide may have additional pre-synaptic effects on serotonin uptake or release.

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## NEURAL PLASTICITY IN ADULT ANIMALS III

269.1 GM1 GANGLIOSIDE REVERSES THE BEHAVIORAL DEFICITS FOLLOWING COLCHICINE-INDUCED GRANULE CELL LOSS IN RAT HIPPOCAMPUS. D. F. Emerich*, D. E. Schmechel* and T. J. Walsh* (SPON: R. Fanelli). NIH/NIEHS, Res. Tri. Pk, NC, 27709 and VA Medical Center, Durham, NC.

GM1 ganglioside exerts a trophic effect on damaged peripheral nerves and also promotes recovery of function following various forms of neural insult. The studies presented here examined the effects of GM1 on the behavioral and morphological alterations induced by intradentate injections of colchicine (COL). This treatment selectively destroys granule cells in tbe hippocampus and produces persistent alterations in both cognitive and motor behaviors.

Three days prior to surgery and for 25 days thereafter Fischer rats were injected i.p. with 30 mg/kg GM1 or saline (vehicle). COL (3.5 ug site) or saline was bilaterally injected into the dentate gyrus at two rostrocaudal locations. Rats injected with COL (COL-SAL) were significantly more active than the controls (SAL-SAL) 7, 14, 21 and 30, but not 39, days after surgery. In contrast, the COL group treated with GM1 (COL-GM1) exhibited a transient hyperactivity which was only evident 7 days after surgery. Thus, GM1 enhanced the rate of recovery of normal motor behavior following COL. COL also impaired retention of a passive avoidance response 14 days post treatment. The COL-SAL group had significantly shorter retention latencies 48 hrs following training, while the COL-GM1 group exhibited no deficits in this task. In a final series of studies, the COL-SAL group exhibited altered responsiveness to a variety of psychoactive drugs. They were more sensitive to (a) the motor stimulant effects of apomorphine, and (b) the analgesic effects of morphine. However, they were less sensitive to the motor activating effects of scopolamine. GM1 prevented all of these alterations in pharmacological responsiveness.

After behavioral analysis, rats were perfused with 4 % paraformaldehyde-0.1 % glutaraldehyde for histology. The COL-SAL group showed a loss of granule cells, mild ventricular dilitation, and a decrease of hippocampal volume, with relative preservation of pyramidal neurons in other subfields and basket cells in the dentate gyrus. The neural damage was present, but clearly minimized in the COL-GM1 group.

Thus, the effects of GM1 on COL-induced behavioral and morphological changes might provide a useful model system for studying neurodegenerative disorders and their treatment.

269.2 **PURIFICATION OF A KINASE C SUBSTRATE: BRAIN PHOSPHOPROTEIN F1 AND THE DISCOVERY OF AN ENDOGENOUS KINASE C INHIBITORY FACTOR.** **S.Y. Chan, K. Murakami* and A. Routtenberg. CRESAP Neuroscience Laboratory, Northwestern University, Evanston, Ill. 60201.**

**The *in vitro* phosphorylation of Protein F1 ($M_r$=47kD, pI=4.5) was selectively increased and directly related to synaptic plasticity of long-term potentiation [*Behav Neuro Biol*, (1985), 43, 3-11,]. Recent evidence using crude synaptosomal plasma membranes suggested that phosphorylation of protein F1 was stimulated in a dose-dependent manner by the additon of a partially purified kinase C (PKC) fraction [*Brain Res*, (1985), in press].**

**We have begun protein F1 purification to determine whether F1 is a substrate for protein kinase C under controlled reaction conditions. Protein F1 of high purity was isolated using an osmotically-shocked $P_2$, membrane fraction as starting material. F1 was extracted from the membrane fraction using first pH 11.5, then pH 5.5 buffer. Further purification by hydroxylapatite (HP) column eluted with 30-75mM KPi (pH 7.2), then P-100 gel filtration chromatography, yielded two bands of 67kD and 47kD in silver-stained 10% SDS gels. Isoelectrofocusing (2D gel) showed that the 47kD protein was acidic (pI=4.5), similar to the known physical properties of protein F1 in $P_2$,. This is the first demonstration that a highly purified protein F1 can be phosphorylated by the addition of a highly purified PKC (see Murakami *et al.*; this meeting). This phosphorylation was absolutely dependent on phosphatidylserine (PS, $K_a$=40ug/ml) and $Ca^{2+}$ ($K_a$=50uM). 1,2 diolein reduced the $K_a$ of free $Ca^{2+}$ for F1 phosphorylation by exogenous PKC to 10uM in the presence of PS.**

**During the isolation of protein F1, we have discovered an endogenous inhibitory factor of F1 phosphorylation. This factor could not be precipitated by 80% $(NH_4)_2SO_4$ and its activity was destroyed by trypsin (10ug/ml, 30 min. at 30°C), suggesting that it is a protein. The inhibitory factor has been partially purified using the HP column, eluted with 200-400mM KPi, pH 7.2. A 50% inhibition of F1 phosphorylation by PKC was observed using this fraction (dialysed in 50mM Tris). Similar inhibition of phosphorylation was found when exogenous histone H1 instead of F1 was used as PKC substrate. This inhibitory factor may thus be an endogenous inhibitor of kinase C and could regulate the PKC-F1 dependent synaptic plasticity. (Supported by MH 25281-12 and AFOSR 83-0335 to A.R.).**

269.3 **PHORBOL ESTER, WHICH INDUCES PROTEIN KINASE C (PKC) TRANSLOCATION TO THE MEMBRANE, PREVENTS DECAY OF LONG-TERM POTENTIATION** D. Lovinger*, P. Colley*, D.J. Linden*, K. Murakami*, and A. Routtenberg. Cresap Neurosci. Lab., Northwestern Univ., Evanston, IL 60201.

Long-term potentiation (LTP) in the hippocampal formation induces translocation of PKC activity to the membrane [*Fed Proc* (1985) 44 1421], and increases Protein F1 phosphorylation [*Behav Neural Biol* (1985) 43 3-11] as do phorbol esters and exogenous PKC [Akers et al., *Br Res* (1985) in press]. To test the hypothesis that increased PKC translocation alters LTP we compared potentiation in the hippocampus of the intact rat following local application of phorbol myristate acetate (PMA), a compound which induces PKC translocation [*Nature* (1983) 301 621-623], or 4-alpha phorbol (4AP), an inactive phorbol compound.

In urethane anesthetized rats a stimulating electrode was placed in the perforant path, and a 5-barrelled micropipette for iontophoretic application of PMA or 4AP was placed to record field potentials from the hilus of the dentate gyrus. Following drug application both low (0.2 Hz) and high (two 8 pulse trains at 400 Hz) frequency stimulation were given.

LTP exhibited less decay after PMA than after 4AP; Fig 1 shows that the PMA group retained a greater percentage of inital potentiation of population spike amplitude (SA) 30-60 min after high frequency stimulation than the 4AP group. Application of PMA did not affect responses to low frequency stimulation prior to LTP. Thus PMA alone appears incapable of inducing synaptic plasticity. PMA at 30ng/ml reduced the Ca2+ requirement for activition of purified PKC (see Murakami et al., this meeting) from $K_a$~30uM to $K_a$~2uM. PMA may act synergistically with molecular events induced by high frequency stimulation (eg. elevated intracellular calcium) to activate PKC and promote and prolong synaptic plasticity. (This work was supported by MH 25281-12, and AFOSR83-0335 to A.R).

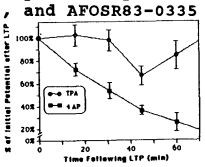



Fig 1. Decay of potentiation was calculated using the formula: (% increase in SA vs. baseline at time x)/(% increase in SA immediately following LTP). Values are means ± SEM. N=6 in both groups.

269.4 **A PHOSPHOPROTEIN (F1) DIRECTLY RELATED TO NEURAL PLASTICITY IN ADULT RAT BRAIN MAY BE IDENTICAL TO A MAJOR GROWTH CONE MEMBRANE PROTEIN (pp46).** R.B. Nelson*, A. Routtenberg, C. Hyman*, K.H. Pfenninger*. (SPON: R. Sekuler) Cresap Neuroscience Laboratory, Northwestern University, Evanston, IL, and Dept. Anatomy and Cell Biology, Columbia University P and S, New York, NY.

Protein F1 and a protein F1 kinase have been implicated in the molecular regulation of neural plasticity: F1 phosphorylation is increased in direct relation to the production of long-term potentiation [LTP; *Behav.Neur.Biol.*(1985)43:3], a physiological model for synaptic plasticity. Concomitantly, activity of a phospholipid/$Ca^{2+}$-dependent protein kinase, which phosphorylates protein F1 (Akers and Routtenberg, *Br.Res.*,in press), is translocated from cytosol to membrane [*Fed.Proc.*(1985)44:1421]. The increase in F1 phosphorylation also accompanies LTP lasting 3 days (Lovinger *et al.*,*Br.Res.*,in press).

Phosphoproteins of nerve growth cones isolated by subcellular fractionation from fetal brain ["growth cone particles"; *Cell*(1983)35:573] have recently been characterized (Katz *et al.*, *J.Neurosci.*,in press). We report here that protein F1 may be identical to pp46, a major membrane-bound growth cone phosphoprotein, on the basis of molecular mass ($M_r$ ~47kD), isoelectric point (~4.4), microheterogeneity, and phosphopeptide maps following limited proteolysis with *S. aureus* V8 protease (fragments of 23kD, 13kD, and 11kD). F1 and pp46 are both phosphorylated by a $Ca^{2+}$/calmodulin-dependent kinase. While F1 phosphorylation is stimulated by phosphatidylserine and phorbol ester (TPA), the phosphorylation of its counterpart in growth cone particles is stimulated by TPA, but by phosphatidylserine only under certain conditions.

Phosphoproteins and their kinases which are enriched in growth cones may be involved in the regulation of neurite growth in brain development. In adult brain, growth of presynaptic terminals has been observed in model systems of neural plasticity [*Science* (1972)176:191]. Thus, the fact that LTP enhances phosphorylation of a protein similar, if not identical, to one characteristically found in growth cones suggests that a common mechanism underlies both normal neurite growth and the generation of LTP. (Supported by MH25281-12 and AFOSR 83-0335 to A.R., NIH NS 21729 to K.H.P., NRS fellowship DA05254 to R.B.N., and an MDA fellowship to C.H.).

269.5 **DIRECT STIMULATION OF PROTEIN KINASE C (PKC): UNSATURATED FATTY ACIDS (OLEATE, ARACHIDONATE) ARE SUFFICIENT.** K. Murakami*, S.Y. Chan, and A. Routtenberg. Cresap Neurosci. Laboratory, Northwestern University, Evanston, IL 60201 (SPON: J.P. Rosenfeld).

Protein F1 (Mr: 47K, pI: 4.5) phosphorylation state is changed by long-term potentiation (LTP; *Behav. Neural Biol.* (1985) 43:3). F1 is a substrate of PKC (*Brain Res.* (1985) in press). LTP in hippocampus induces the translocation of PKC activity from cytosol to membrane, the active site of PKC (*Fed. Proc.* (1985) 44:1421), suggesting an important role for PKC in synaptic plasticity.

It has been shown that diacylglycerol (DAG), containing unsaturated fatty acid (UFA) in the acyl position greatly increases the affinity of PKC for $Ca^{++}$. DAG may be a second messenger for PKC activation. We have examined whether the UFA itself of DAG directly stimulates PKC. PKC was purified to apparent homogeneity (700-1000 fold) by a 4-step LC procedure: 1) DEAE-cellulose 2) AcA34 gel filtration 3) hydroxytapatite and 4) phenyl-sepharose chromatograghy. Homogeneity was confirmed by SDS gelelectophoresis.

Two unsaturated fatty acids were evaluated. Oleic acid ($18:1^{\Delta 9}$) and arachidonic acid ($20:4^{\Delta 5,8,11,14}$) directly stimulated PKC *without phospholipids* in the presence of $Ca^{++}$ at a $K_a$ of 50 uM (oleate) and 53 uM (arachindonate). $V_{max}$ for oleic acid was similar to that for phosphatidylserine (PS). In contrast to UFA, saturated fatty acid like stearic acid did not stimulate PKC. Methyl esters of UFA also had no effect on PKC activation.

PKC may be *phospholipid-sensitive* rather than phospholipid dependent since oleic or arachadonic acid alone was sufficient to activate PKC. One possible mechanism is that phospholipases release UFA from phospholipids and free UFA stimulate PKC translocation/activation directly. This is supported by the fact that oleic acid stimulates protein F1 phosphorylation using a partially purified F1 substrate (see Chan *et al.*, this meeting). Alternatively, an arachidonate and oleate at acyl position in phospholipids are actually involved in the physiological activation of PKC. These new findings suggest a potential role of oleic or arachadonic acid in synaptic plasticity. (Supported by MH 25281-12 and AFOSR 83-0335 to A.R.).

269.6 EFFECTS OF INTRACEREBROVENTRICULAR KAINIC ACID ON THE PERFORANT PATH PROJECTION TO THE RAT FASCIA DENTATA. David R. Armstrong, Debra A. Evenson* and J. Victor Nadler. Dept. Pharmacology, Duke Univ. Med. Ctr., Durham, NC 27710.

Intracerebroventricular (i.c.v.) administration of kainic acid (KA) destroys neurons of the hippocampal CA3-CA4 area, but spares dentate granule cells and cells of origin of the perforant path (PP). A previous study showed that, although PP fibers do not degenerate after bilateral i.c.v. administration of KA, nevertheless the density of PP innervation in the dentate molecular layer is transiently reduced. Dentate granule cells were suggested to shed excitatory innervation in response to destruction of the CA3-CA4 neurons to which they project. The present study was undertaken to determine whether this reversible loss of PP synapses implies a depression of PP-evoked granule cell activation.

Male rats were prepared unilaterally with an indwelling i.c.v. cannula, a bipolar stimulating electrode in the angular bundle and a microdrive mounted on the skull above the hippocampus. After a 2-wk recovery period, a tungsten recording electrode was lowered into the dentate hilus, and "input-output" curves (field EPSP vs. stimulus current and population spike vs. field EPSP) were generated. KA (1.41 nmol) was infused through the cannula and input-output curves were obtained every other day for two weeks. A separate series of animals was similarly infused with KA and prepared for electron microscopy. All subjects treated with KA developed relatively complete lesions of the ipsilateral CA3 area, with variable involvement of the ipsilateral CA4 and minimal cell death in the contralateral hippocampus.

I.c.v. infusion of KA transiently reduced the maximum amplitude of the PP-evoked field EPSP and population spike. Simultaneously, the excitability of the granule cells appeared to have increased, as suggested by a leftward shift of the population spike/EPSP curve with no comparable change in the EPSP/current relationship. These effects were maximal 2 d after the infusion and reversed by day 8. In some rats spontaneous epileptiform discharges were recorded during the postinfusion period, and the occurrence of these ictal events altered evoked synaptic activity.

Electron microscopic studies revealed that unilateral infusion of KA reduced the density of PP innervation in both ipsilateral and contralateral dentate molecular layers. This reduction appeared to be maximal (~25%) 2 d after the infusion and essentially reversed by day 8. Thus the synaptic deficit may account, in part, for transient depression of the maximal electrophysiological response. However, the destruction of target cells does not appear to act as the stimulus for these effects, since KA reduced PP synaptic density bilaterally whereas only ipsilateral granule cells were extensively deprived of their postsynaptic targets. (Supported by NIH grant NS 06233.)

269.7 INVOLVEMENT OF MUSCARINIC CHOLINERGIC RECEPTORS IN KINDLING. V. Westerberg* and M. E. Corcoran. Department of Psychology, University of Victoria, Victoria, B. C., Canada V8W 2Y2.

A previous report (Westerberg & Corcoran, Neurosci. Abstracts, 1983) indicated that the muscarinic cholinergic antagonist scopolamine slowed the rate of amygdaloid kindling in Sprague-Dawley rats. Two experiments were conducted to further investigate this finding.

In the first experiment, two weeks after implantation of electrodes, male Sprague-Dawley rats received application of unilateral amygdaloid stimulation once every 2 days. Some rats received an IP injection of scopolamine hydrobromide (1, 5, 10, or 15 mg/kg) 15 min before each stimulation, and others received 1.5 ml/kg of the distilled water vehicle. If rats failed to develop bilaterally generalized (stage 5) seizures by the cutoff of 16 afterdischarges (ADs) they were given a 14-day rest without stimulation and then rekindled without the drug. Scopolamine slowed the rate of kindling in a dose-related manner with increased doses of scopolamine resulting in increased seizure prophylaxis (control, M=11.78, SEM=1.21; 1mg/kg, M=12.33, SEM=1.34; 5mg/kg, M=20.25, SEM=3.73; 10mg/kg, M=18.22, SEM=2.05; 15mg/kg, M=22.20, SEM=2.85). Of the 36 rats treated with scopolamine, 21 failed to develop past unilateral clonic seizures by the cutoff, and required as many additional drug-free ADs to rekindle (M=11.07, SEM=2.08) as controls required for initial kindling. This suggests that scopolamine can produce a genuine prophylactic effect, in agreement with the findings of Arnold et al., (Exp. Neurol., 1973, 40, 457) with another muscarinic antagonist, atropine. Scopolamine administered to kindled rats did not affect the intensity or duration of previously kindled seizures, indicating that scopolamine does not act like an anticonvulsant.

In the second experiment Sprague-Dawley rats were treated as above except that they were given the distilled water vehicle, 10 mg/kg scopolamine hydrobromide, or 10.36 mg/kg scopolamine methyl bromide, the quaternary derivative of scopolamine that does not easily cross the blood-brain barrier. Scopolamine again slowed seizure development (control, M=10.00, SEM=1.85; scopolamine M=23.87, SEM=4.75; methyl scopolamine, M=15.20, SEM=1.66) compared to both the control and methyl scopolamine-treated rats. Methyl scopolamine-treated rats kindled at the same rate as controls. Methyl scopolamine given to kindled rats did not affect the intensity or duration of established seizures.

The present results support the hypothesis that central cholinergic or cholinoceptive neurons play an important role in the development of kindled seizures, but that once seizures have developed, cholinergic neurons are no longer involved.

Supported by MRC and NSERC.

269.8 CHANGES IN CENTRAL MONOAMINES AFTER AMYGDALOID KINDLING. J. Lewis*, V. Westerberg* and M. E. Corcoran (SPON: R. Skelton). Department of Psychology, University of Victoria, Victoria, B. C., Canada.

There is considerable evidence that central monoamines (MAs) can modulate the rate of kindling. Some of this evidence is correlative, with some studies finding changes in MAs after kindling and other studies finding no changes. The discrepancies between the different studies may be attributable to variations in biochemical procedures, numbers of seizures evoked, and the intervals between the last seizure and time of sacrifice. We reexamined the question by holding constant the procedures for kindling and postmortem biochemistry while varying the intervals at which rats were killed after the last seizure.

Male hooded rats received bilateral implantation of electrodes into the amygdala. Some rats were stimulated in one amygdala until 3 generalized seizures were kindled; other rats served as yoked controls and received an equal number of nonconvulsant 3-Hz stimulations. Rats were killed by cervical fracture either 2 weeks or 4 weeks after the last seizure. The brains were rapidly dissected over ice, tissue was homogenized and frozen at -196°C, and regional concentrations of MAs (and in some cases major metabolites) were later determined using HPLC with electrochemical detection.

Changes in noradrenaline (NA): We found a small but significant depletion of NA in the ipsilateral frontal cortex at 2 weeks, but this had recovered to normal values at 4 weeks. A small but significant depletion of NA was detected in contralateral hippocampus at 4 weeks; there was no corresponding change in MHPG, a major metabolite of NA. There were no other changes in NA.

Changes in dopamine (DA): There were no significant changes in DA.

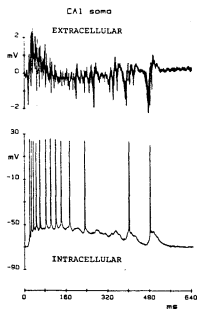
Changes in serotonin (5-HT): There were small but significant depletions of 5-HT in the contralateral hypothalamus and hippocampus at 2 weeks. The hippocampal depletion was accompanied by a comparable decline in 5-HIAA, the major metabolite of 5-HT. These changes were no longer detectable at 4 weeks, whereas a significant depletion of 5-HT was observed in the ipsilateral hippocampus at 4 weeks, accompanied by a nonsignificant increase in 5-HIAA. There was a significant increase in 5-HIAA in the ipsilateral hypothalamus, accompanied by a nonsignificant depletion of 5-HT, at 4 weeks.

We failed to replicate most previous reports of changes in MA concentrations in kindled brain. We did find some changes in MAs or metabolites, but these were not consistent across the 2 intervals. Thus the alterations in MA concentrations produced by kindling do not fall into a simple and readily interpretable pattern.

Supported by MRC and NSERC.

269.9 REDUCED INHIBITION AND EPILEPTIFORM DISCHARGES IN THE IN VITRO HIPPOCAMPUS PRODUCED BY 3-MERCAPTOPROPIONIC ACID (3-MPA). A.Stelzer*, N.T.Slater and G.ten Bruggencate. Physiology Institute, Univ. Munich, Pettenkoferstr. 12, D-8000 Munich 2, W. Germany.

The actions of many convulsant and anticonvulsant agents have been related to their ability to reduce or facilitate GABA-mediated ipsps in the hippocampus and other structures. In the present experiments we have examined the effects of the convulsant agent 3-MPA, a competitive inhibitor of the GABA synthesizing enzyme glutamic acid decarboxylase (GAD), on excitatory and inhibitory postsynaptic potentials in the guinea-pig hippocampal slice. Slices were cut on a vibrating tissue cutter and maintained at 30-33°C in Krebs Ringer of the following composition (mM): NaCl 118, KCl 3, $NaHCO_3$ 25, $NaH_2PO_4$ 1.2, $MgCl_2$ 1, $CaCl_2$ 1.5, D-glucose 11. Intra- and extracellular recordings were made in the CA1 stratum pyramidale of slices in which the CA2/3 subfield was removed by dissection.Evoked responses were produced by stimulation of both stratum radiatum and alveus. The bath application of 1 X $10^{-6}$ to 2 X $10^{-5}$M 3-MPA reversibly induced both orthodromically-evoked and spontaneous (see Fig) epileptiform discharges after exposures of 60-90 min. This concentration of 3-MPA had no significant effect on the population spike and intra- and extracellularly recorded epsp, but produced a complete abolition of both anti- and orthodromically-evoked ipsps.




This reduction of ipsps was not associated with alterations of the reversal potential or membrane conductance. Seizure discharges appeared 20-30 min after the complete blockade of ipsps. Higher doses of 3-MPA (> $10^{-4}$M), however, rapidly and reversibly reduced both epsps and ipsps within 1-2 min of application, and no seizure discharges were observed at these concentrations. The reduction of synaptic potentials in the presence of high doses of 3-MPA was associated with an increase of membrane conductance, but no significant change in membrane potential.

These results therefore demonstrate that GABA-mediated ipsps in the hippocampus can be reduced by a competitive inhibitor of GABA synthesis in a concentration range that closely corresponds to the $K_i$ for in vitro inhibition of GAD (1.8 $\mu M$[1]). Furthermore, these experiments suggest that either a considerable safety factor exists for the inhibitory control of epileptiform discharges, or the convulsant effects of 3-MPA may result from some other action of the drug.

(Supported by Sonderforschungsbereich 220, Teilprojekt B2).

[1]Wu,J.-Y., In 'GABA in Nervous System Function', Raven Press, 1976.

269.10 CHRONIC DESIPRAMINE TREATMENT RETARDS THE DEVELOPMENT OF KINDLED SEIZURES FROM ENTORHINAL CORTEX IN RAT. C. Applegate* and and J.L. Burchfiel (SPON: C.T. Lombroso). The Children's Hospital, Boston, MA 02115.

Norepinephrine (NE) systems have been suggested to play an inhibitory role in the development of kindled seizures. Much of this evidence is derived from depletion studies in which the removal of NE results in a facilitation in the rate of development of generalized seizures. The chronic administration of the NE uptake inhibitor desipramine (DMI) has been shown to result in reductions in both NE beta-receptor numbers and isoproterenol-stimulated adenylate cyclase activity which endure for a period of time following drug withdrawal. We have employed chronic DMI treatment as a means of altering NE system functioning to further examine the role of NE in generalized seizure development during kindling. We predicted that this treatment would decrease beta-receptor functioning and concommitantly facilitate the rate of kindling.

Adult, male, Sprague-Dawley rats were unilaterally implanted with bipolar stimulating electrodes into the entorhinal cortex, in or near the area of the angular bundle. Following recovery, animals received intraperitoneal injections of either DMI (15mg/kg) or saline daily for 7 days. Two days following drug treatment, after-discharge (AD) thresholds were determined and kindling trials were begun. Animals received twice daily electrical stimulation (ISI > 2 hrs.) at suprathreshold (+50 - 100μA) current levels. Rats were stimulated until a minimum of 6 consecutive fully generalized seizures were elicited. It is important to note that animals were never electrically stimulated in the presence of DMI in this experimental design.

Contrary to our expectation, chronic DMI treatment significantly increased the number of stimulations required to elicit the first fully generalized seizure as well as the number of stimulations required to reach our criterion of 6 consecutive generalized seizures (median trials to criterion: DMI=110; saline=45). This effect occurred in the absence of differences in threshold current levels for the elicitation of AD's or AD durations.

These data suggest that the relationship between a down-regulated NE beta-receptor system and the development of kindled seizures is complex and may involve additional adaptive mechanisms.

Supported by NIH grant NS20351.

269.11 KINDLING ANTAGONISM: THE EFFECTS OF NOREPINEPHRINE DEPLETION USING DIFFERENTIAL 6-OHDA REGIMENS. J.L. Burchfiel, R. Konkol* and C. Applegate*. Children's Hospital, Boston MA 02115.

The concurrent, alternate stimulation of limbic system structures can result in a relative or absolute suppression of the expression of fully generalized seizures at one or both of the stimulated sites. This is a phenomenon we have labeled "kindling antagonism" (Burchfiel, et al., Exp. Neurol., 75:479-489 (1982)). We recently have been interested in the mechanism of inhibition of seizure suppression at the antagonized site, and have begun a series of experiments aimed at defining a role for norepinephrine (NE) in the kindling antagonism phenomenon.

Male, Sprague-Dawley rats were administered 6-OHDA in two regimens designed to produce different patterns of brain NE depletion: (1) adult rats were administered 25 ug of 6-OHDA intracisternally 3 times at equally spaced intervals over 7 days, and (2) neonatal rats were administered a single intracisternal injection of 15 ug of 6-OHDA within 24 hrs of birth. Following a 14 day recovery period (regimen 1) or at maturity (regimen 2) rats were implanted with bipolar stimulating electrodes into the septal nucleus and ipsilateral entorhinal cortex and were introduced into the kindling antagonism paradigm. Following kindling, biochemical (HPLC-EC) and histochemical (glyoxylic acid) analyses were performed to assess the effect of the neurotoxin treatments.

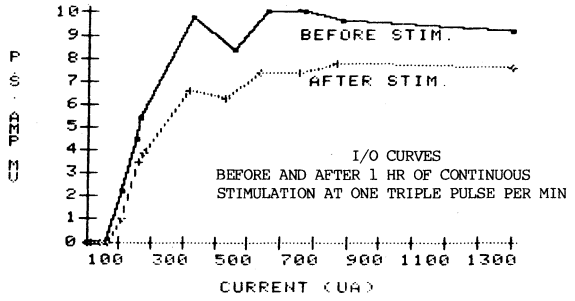
Adult 6-OHDA treatment resulted in significant whole brain reductions in NE and almost completely eliminated the development of kindling antagonism. Neonatal treatment also resulted in forebrain reductions in NE, but produced brainstem and cerebellar increases in NE levels and hindbrain neuronal hyperinnervation. In contrast to adult treatment, neonatal treatment left the development of kindling antagonism intact. Neither 6-OHDA regimen altered the threshold for the elicitation of afterdischarges or the afterdischarge duration. In addition, neither regimen produced reductions in striatal dopamine or dopamine metabolite levels.

These results suggest an inhibitory role for NE in the expression of generalized seizures from the suppressed site in this model and further suggest the involvement of pontine, medullary and/or cerebellar circuitry in the kindling antagonism phenomenon.

Supported by NIH grant NS20351.

269.12 ALTERATION OF HIPPOCAMPAL DENTATE FIELD POTENTIALS ELICITED BY LOW FREQUENCY STIMULATION. J.B.Wiesner, S.J.Henriksen, and F.E.Bloom, Div. of Preclinical Neuroscience and Endocrinology, Scripps Clinic and Research Foundation, La Jolla, Ca. 92037.

Electrical stimulation of the perforant pathway of the hippocampus elicits complex "field potentials" that can be recorded from the dentate gyrus. As a part of our neuropharmacological analyses of these evoked events we have stimulated this pathway for prolonged periods of time (1-4 hrs) in halothane-anesthetized rats. Field potentials were recorded in the granule cell layer via single-barrel glass micropipets. Stimulation paradigms involved sets of single, double, or triple constant-current pulses continuously delivered to the ipsilateral angular bundle of the perforant path, at intervals ranging from 10 to 60 sec. In accordance with reports by others using both in vivo and in vitro preparations (Brain Res. 110:143,1976 and 151:623,1978), we observed that the initial PS and the subsequent potentiated PS of multi-pulse stimulations varied in amplitude over time, particularly at higher rates of stimulation (i.e. $\geq$0.1 Hz). However, we have also observed significant alterations in evoked PS amplitudes when much slower rates of stimulation were extended over long periods of time. Such alterations of PS amplitude include: (1) long-term decreases; (2) long-term increases; and (3) transient fluctuations of varying duration. Long-term decreases were a predominant effect, and were marked by a decrease of up to 60% in the maximal response in stimulus-response curves (e.g. illustrated below). The lack of stability of PS amplitude, even with very low rates of stimulation, must be an essential consideration in medium- or long-term pharmacological tests in both in vivo and in vitro hippocampal preparations. The bases of these long-term changes are under investigation. (Supported by AA 06420 and DA 03665).




269.13 OLFACTORY BULB KINDLING: EFFECTS ON FIELD POTENTIALS EVOKED IN THE OLFACTORY BULB, PYRIFORM CORTEX, DENTATE GYRUS, AND RETICULAR FORMATION. R. D. Russell and J. S. Stripling. Department of Psychology, University of Arkansas, Fayetteville, AR 72701.

This study investigated the anatomical generality of effects of olfactory bulb (OB) epileptiform afterdischarge (AD) and kindling on OB-stimulated field potentials. Male Long-Evans rats (N=17) were implanted with recording electrodes in the OB, anterior or posterior pyriform cortex (PC, a monosynaptic OB projection site), dentate gyrus (DG, a polysynaptic OB projection site), and reticular formation (RF). Rats received one of the following experimental treatments: 1) daily AD via 50 pulse/sec electrical stimulation of OB (KINDLED group, N=7); 2) daily 1 pulse/sec stimulation (SHAM group, N=5); or 3) daily acclimation to the recording chamber (nonstimulated CONTROL group, N=5). Evoked potentials (EPs) were collected from the KINDLED and SHAM subjects immediately preceding each experimental treatment and from all subjects 3 and 10 days following the last treatment.

Kindling differentially affected late components of the EPs in the OB and DG. For example, in the OB a persistent increase in a late and long duration (> 60 msec) negative wave was associated with kindling whereas an earlier positive wave was unaffected. In the DG, kindling was associated with a large (> 400%) and persistent increase in a late positive wave (30-45 msec latency) whereas an earlier positive wave (15-25 msec latency) and later negative wave were unaffected. Although quantitative differences in the PC potential were evident between anterior and posterior sites, kindling's effects were relatively homogeneous across these placements. These effects, consistent with those reported earlier (Russell and Stripling, Soc. Neurosci. Abstr. 10: 187, 1984; Brain Res., in press), included an interesting pattern of changes in the population EPSP component of the EP. Early in kindling, the population EPSP in the PC was increased in amplitude whereas it declined to baseline levels later in kindling development. In the RF, kindling was associated with an asymptotic 400% increase in the amplitude of a long-latency negative component.

In summary, OB kindling is associated with anatomically extensive plasticity which is indicative of altered responsiveness in numerous sites to synchronous activation of the OB. Some of these effects (e.g., the component-specific effect in the DG) are difficult to explain solely as a result of enhanced excitability of OB mitral cells.

269.14 BENZODIAZEPINE ANTAGONISTS AND THE AMYGDALOID KINDLED SEIZURE IN RATS. T.E. Albertson and W.F. Walby*. Dept. of Internal Medicine, Univ. of Calif., Davis, CA 95616.

The anticonvulsant effectiveness of the benzodiazepine antagonists RO15-1788 (RO), CGS-8216 (CGS), and PK-11195 (PK) disolved in DMSO were evaluated against threshold and suprathreshold stimulation in previously amygdaloid kindled male Sprague-Dawley rats. Using a double injection paradigm, the effectiveness of these three antagonists to reverse the anticonvulsant properties of 3mg/kg of diazepam were also studied in this model. Pretreatment with RO (3, 10, and 30mg/kg) reduced, though not statistically significant, the afterdischarge duration (80.5% control) and the seizure rank (3.8 compared to control of 5) at the highest dose tested after suprathreshold stimulation. Pretreatment with either CGS (3, 10, and 30mg/kg) or PK (10 and 60mg/kg) failed to consistently modify either elicited afterdischarge durations or seizure ranks. Afterdischarge thresholds were also not consistently modified by pretreatment with either of the three antagonists.

In the double injection paradigm, 3mg/kg diazepam significantly reduced both elicited afterdischarge duration and seizure rank when injected 15 minutes before or after a second injection of control DMSO (0.25cc/kg) before suprathreshold stimulation (30 minutes from first injection to stimulation). When either CGS (10mg/kg) or RO (10mg/kg) were given 15 minutes before 3mg/kg diazepam prior to stimulation, the anticonvulsant properties of diazepam were blocked. When CGS (10mg/kg) and PK (10mg/kg) were given 15 minutes after 3mg/kg diazepam, the anticonvulsant effectiveness of diazepam was not blocked. However, RO (10mg) given 15 minutes after diazepam (3mg/kg) effectively blocked the anticonvulsant properties of diazepam. Increasing the dose of PK to 60mg/kg 15 minutes after diazepam failed to block the anticonvulsant effectiveness of 3mg/kg diazepam. When 10mg/kg of CGS was given less than 10 minutes after diazepam or if the interval was held at 15 minutes after diazepam but the dose was increased to 30mg/kg of CGS, the anticonvulsant effectiveness of diazepam was blocked. Together, these data point to the lack of significant anticonvulsant effectiveness of these three benzodiazepine antagonists in the fully kindled amygdaloid model of epilepsy in the rat. Both RO and CGS have demonstrated the ability to reverse the anticonvulsant effectiveness of diazepam when given before or after diazepam dosing as long as appropriate dose and dosing intervals of CGS are used. High doses of PK failed to reverse the anticonvulsant effectiveness of diazepam when tested after diazepam dosing in this model. (Supported in part by BRS-2507RR 05684-16)

269.15 THE ASYMPTOTIC LIMITS OF LONG-TERM POTENTIATION/DEPRESSION ARE INDEPENDENTLY CONTROLLED. H.S.LOPEZ*, B.BURGER & W.B LEVY (SPON: M.Merickel).Dept. Neurosurgery, Univ. of Va. C'ville, VA 22908.

An algebraic description of synaptic modification consistent with our work at the entorhinal cortex-dentate gyrus (EC-DG) synapses is $m_i = e \cdot f(y) \cdot (x_i - cm_i)$. e represents the contribution of nonspecific inputs; f(y) refers to an integrative postsynaptic event specific at least on a cell by cell basis and probably specific to a postsynaptic dendritic domain; and $(x_i - cm_i)$ provides the specificity that allows individual modification of individual synapses. $(x_i - cm_i)$ also controls the asymptotic limits of potentiation and depression when $e \cdot f(y)$ is non-zero. The data here agree with this formulation and emphasize the individuality of synaptic modification, particularly the individuality of the asymptotic limits of synaptic modification.

The experiments resemble (1) except for tetrodotoxin injections into the EC to reduce spontaneous activity. Stimulating electrodes were placed in the angular bundles (AB). Field potentials (pEPSP) were monitored in the DG on both sides, recording a contralateral and ipsilateral pEPSP for each AB stimulation. 9 rats received 2 distinct conditioning periods (CPs) separated by a 15' test period. In both CPs, conditioning consisted of 30 trains of 8 pulses @400Hz (1 train/145s). These bilateral trains were asynchronous with the train to one AB (trailing AB) coming 3 ms after the train to the other AB (leading AB). In CP2 the asynchrony was reversed with the leading AB becoming the trailing. One hour of testing followed CP2. All test stimuli are single unilateral pulses.

With either CP the leading contralateral potentiates and the trailing one depresses, i.e. a contralateral afferent synapse potentiates and then depresses, or vice versa, over the 2 CPs. Important here are results after CP2 since both ipsilateral responses were taken to asymptotic potentiation by CP1 (after CP1, the ipsilateral pEPSP increased 63% of its preconditioning value while only 15% after CP2). After CP2 the pEPSP of the CP2-leading contralateral increased 97% of its prior value while the CP2-trailing contralateral decreased to 31% of its prior value.

Thus, by driving ipsilateral afferents to asymptotic potentiation, contralateral potentiation and depression are shown to be independent of either converging or collateral ipsilateral changes. Further, both contralateral potentiation and depression are independent of converging and collateral effects in the sense that they are not subject to the process that produces asymptotic limitations upon either group of ipsilateral afferents. These results agree with an algebraic formulation in which individual synapses are individually modifiable and the parenthetical "presynaptic term" $[(x_i - cm_i)]$ controls asymptotic changes on a synapse by synapse basis. (1) Levy & Steward, Neurosci. 8(1983)799.Supported by AFORS 83-0236 and NIH NS15488 to WBL.

269.16 THE DEVELOPMENT AND DECAY OF KINDLING-INDUCED INCREASES IN PAIRED-PULSE DEPRESSION IN THE DENTATE GYRUS. M. deJonge* and R.J. Racine, Department of Psychology, McMaster University, Hamilton, Ontario, Canada L8S 4K1.

Inhibition in the hippocampal dentate gyrus (DG) of the rat has been shown to increase as a result of kindling, possibly due to an increase in benzodiazepine (Bz) receptors (Tuff et al., Brain Res. (277)1983(79). The kindling-induced increase in Bz receptors appears to be inversely related to time since the last stimulation, so it is expected that the increase in inhibition also decays over time. The present study investigated the time course, for both development and decay, of the increase in inhibition due to kindling. Rats were kindled in either the DG or in the perforant path, the main afferent pathway to the dentate gyrus. Animals in the control group received no kindling stimulation but were treated identically otherwise. Paired-pulse (P-P) and input/output (IO) measures (of responses evoked in the DG by pulses applied to the perforant path) were taken 24 hrs after the 1st epileptiform after discharge (AD) and 24 hrs after every 9 ADs (3 ADs were triggered each day, 3 hrs apart) until a Stage 5 seizure occurred. The same measures were taken again 24 hrs after the 1st and 4th Stage 5 seizure and 3, 7, 14, and 28 days after the 4th Stage 5 seizure. IO curves revealed that the population spike of the perforant path-kindled animals was potentiated at the higher intensities and depressed at the lower intensities, while DG-kindled animals showed a decrease in spike height over the whole IO curve. Spike height was back to baseline 4 weeks post kindling. Population EPSP (slope) measures in both groups were increased as a result of kindling and continued to rise during the 2 weeks after kindling was terminated. Four weeks after kindling, however, the slope measures had values similar to those observed on the day kindling was terminated. An increase in the early inhibitory component (20-50 msec), as measured by the population spike depression in the P-P experiments, was apparent after the 1st AD. After the 10th AD the late inhibitory component (200-1000 msec) was also increased. No further increase in inhibition was observed after AD 19. The increase in the late inhibitory component decayed back to baseline within 2-4 weeks post kindling while the increase in the early inhibitory component was still apparent 4 weeks post kindling. These results suggest that either the early inhibitory component increases in strength and duration, presumably due to an increase in Bz receptors, or both the early and late components potentiate and decay through independent processes as a result of kindling. In the latter case a biochemical process underlying the changes in the late component needs to be identified. A change in the GABAb receptor system is one possibility.

269.17 KINDLING INDUCES EPILEPTIFORM DISCHARGES AND A PROGRESSIVE ENHANCEMENT OF LONG-TERM POTENTIATION IN THE IN VITRO HIPPOCAMPUS. N.T.Slater, A.Stelzer* and M.Galvan*. Physiology Institute, Univ. of Munich, Pettenkoferstr. 12, D-8000 Munich 2, W.Germany.

It has been proposed that the long-term potentiation (LTP) produced by tetani of hippocampal pathways may underlie the development of epileptogenesis in the kindling model of epilepsy (see McNamara et al., Prog. Neurobiol., 15:139,1980). We have explored this hypothesis using the in vitro hippocampal slice. Guinea-pig hippocampal slices were cut with a vibrating tissue cutter and maintained at 28-32°C in Krebs Ringer of the following composition (mM): NaCl 118, KCl 3, $NaHCO_3$ 25, $NaH_2PO_4$ 1.2, $MgCl_2$ 1, $CaCl_2$ 1.5, D-glucose 11. Stimuli were applied to the stratum radiatum and evoked responses recorded intra- and extracellularly in the CA1 stratum pyramidale. At 30-32°C, repeated tetani (10-20V, 50Hz, 2s delivered 5x at 0.1Hz every 30 min) produced a progressive shift to the left of the relation between stimulus intensity and evoked postsynaptic potential, and a small increase in the maximum evoked population spike. No change in the presynaptic volley was noted. After 4-6 kindling tetani, both spontaneous and stimulus-induced epileptiform discharges appeared, which together with the enhanced orthodromic response, persisted for at least 6 hr. The development of seizure discharges, but not the enhancement of excitability, was suppressed at 28°C. Neither LTP nor the development of seizure discharges were observed when slices were kindled in the presence of the NMDA receptor antagonist ± APV (100μM), which alone had no effect on field potentials. Kindling could then be induced as in control slices after washing out APV. The application of APV to slices already kindled by 4-6 tetani did not abolish the enhanced orthodromically-evoked early potentials, but did reversibly reduce both late extracellularly-recorded afterdischarges and the intracellularly-recorded depolarizing afterpotential.

In the presence of raised Ca (2mM) and Mg (2mM), LTP was observed after the first tetanus, but no further increases of excitability nor epileptiform discharges were induced by subsequent tetani. The progressive enhancement of excitability and the development of epileptiform discharges were also observed in slices in which the CA2/3 subfield was removed by dissection, suggesting that kindling of the CA1 was not dependent on the influence of the CA2/3 'pacemaker' subfield. Measurements of membrane resistance revealed either no significant alteration, or increases following kindling, and no significant alterations of spike accomodation or after-hyperpolarizations were observed.

These results provide support for the hypothesis that repeated kindling-like tetani produce an enhancement of NMDA receptor mediated LTP which may serve as the trigger for the genesis of epileptiform discharges in the CA1 hippocampal subfield.
(Supported by Sonderforschungsbereich 220; Teilprojekt B2).

270.1 Gangliosides GM1 and GD1b ARE ANTIGENS FOR IgM M-PROTEINS IN MOTOR NEURON DISEASE. L. Freddo*, N. Latov* Columbia U., N.Y.,N.Y. 10032, R.K. Yu, Yale U., New Haven, CT.06510, P.D. Donofrio*, H.S. Greenberg*, J.W. Albers*, A.G. Alessi*, A. Leavitt*, G. Davar*, D. Keren*, U. of Michigan, Ann Arbor, MI 48103. (SPON: E.A. Zimmerman)

In patients with plasma cell dyscrasia there may be naturally occurring monoclonal antibodies or M-proteins that have autoantibody activity and cause autoimmune disease. In patients with demyelinating neuropathy and IgM M-proteins for example, the M-proteins bind to a carbohydrate determinant that is shared by MAG and by other glycoproteins and glycolipids in peripheral nerve, and they may cause the neuropathy. We recently studied a patient who resented with lower motor neuron disease and an IgM-lambda M-protein, and found that the M-protein binds to antigens in the central and peripheral nervous system.

Binding of the M-proteins to gangliosides prepared from human spinal cord or cauda equina was examined by immunostaining after separating the gangliosides on high performance thin layer chromatography plates (HPTLC). The plates were coated with polyisobutylmethacrylate in n-hexane, and immunostained with the patient's serum followed by peroxidase-conjugated antibody to human IgM and 3,3'-diaminobenzidine. The IgM immunostained two ganglioside bands in both central and peripheral nerve extracts, which co-migrated with GM1 and GD1b. Immunostaining was specific for the lambda light chain, the same as the M-protein light chain type. Incubation with liposomes containing GD1b or GM1 selectively absorbed the M-protein from the patient's serum, and the serum IgM also immunostained purified GM1 and GD1b on HPTLC plates. Binding of serum IgM to GM1 and GD1b was also tested by ELISA. Microwells were coated with the purified gangliosides, and IgM binding was detected using peroxidase-conjugated antibodies to human IgM and o-phenylenediamine as substrate. Binding of serum IgM to GM1 and GD1b was detectable at seum dilutions of greater than 1:10,000. Control sera, including from patients with IgM M-proteins and neuropathy or normal subjects did not bind to the same glycolipids.

The data suggest that the IgM M-protein in the patient's serum binds to a determinant shared by the gangliosides GM1 and GD1b. The role of the M-protein in causing the lower motor neuron disease is not yet known, but it might bind to one of the gangliosides or to a glycoprotein sharing the same determinant in motor neurons or their axons, and cause neuronal dysfunction and disease.

270.2 THE IMMUNOCYTOCHEMICAL LOCALIZATION OF FUNGI IN THE BRAIN OF AIDS PATIENTS. F. J. Denaro, Dept. of Neuropathology, H-720, UCSD Medical Center, 225 Dickinson Street, San Diego, Cal. 92103

AIDS patients are susceptible to a number of opportunistic infections (OI). Fungal infections figure predominantly among these and are a significant cause of morbidity. The CDC's projected death total for the AIDS epidemic is staggering. Yet, at this time there is no treatment for AIDS. For some time to come, the only course of action is to treat the life threatening OIs. Because an AIDS patient may suffer from many different OIs, and particularly because many of the drugs used to treat such OIs may have adverse side effects; precise diagnosis of the OI is paramount.

The microscopic examination of a buccal smear is sufficient to determine thrush. However, identification of a systemic fungal infection is more difficult. The unique identification of a fungus is not yet possible by serological or immunological means. This is in part due to the complexity of the fungal antigens.(Axelsen, N.H.,Scand. J. Immunol 5,177,1976). Biopsy material is often examined for the suspected pathogen. With this method one may culture or examine the tissue microscopically. Cultures can take time and also the possiblity of retrospective studies are limited. Microscopic examination can be diagnostic, but it also presents a number of pit falls depending on the histologic stains used. For example: The H&E stain and the Fibrin (Weigert)-Gram stains do not stain all fungal elements. If one uses the PAS, one may overlook small fungal cells. The Mucicarmine stain will stain cryptococcus but not other fungi. The GMS is excellent, but it is more involved than the PAP or ABC methods. It would be advantageous to have an immunocytochemical stain for the screening of fungus and for other immunocytochemical studies.

In this study an antibody to Candida albicans (DAKO,#B143) was tested for its immunocytochemical staining ability. This antibody has been used for quantitative immunoelectrophoresis (Axelsen, N.H.,Scand. J. Immunol 5,177,1976) but not immunocytochemistry. It was found that this antibody does indeed stain for Candida as well as staining a number of other fungi. CDC test tissue stained positively for Histoplasma and cryptococcus. Also, one of our controls for Blastomyces stained. This cross-reactivity will be discussed.

In the immunological staining of postmortem brain tissue, candida was found in a number of cortical locations. What appears to be cryptococcus was found in the striatum,cortex and cerebellum.

Acknowledgements: I thank DAKO for the immunocytochemicals and the CDC for test tissues.

270.3 THE BLOOD: BRAIN BARRIER (BBB) IN MURINE HERPES SIMPLEX VIRUS (HSV) MYELITIS: EFFECT OF HIGH DOSE $CO_2$ ON ILLNESS R. R. McKendall, W. Woo*, T. A. Kent*, Depts. of Neurology and Microbiology, University of Texas Medical Branch, Galveston, Texas 77550.

Our earlier observation that systemic antibody administration effectively reduced HSV titers in footpad but not CNS sites of infection suggested that the BBB may prevent antibody access to the CNS during the first several days after infection. To determine if increased BBB permeability would reduce the illness rate, we treated mice with antibody and high dose $CO_2$, which has been shown to reversably increase the blood: brain barrier permeability to large molecules. Preliminary work indicated that mice were able to survive 12% $CO_2$ for 1.5 hours several times a day.

Male, 4 week old mice, n=50, were infected with HSV in the footpad and divided into the following treatment groups: a) No treatment, n=10, b) 0.5cc 1:64 titer anti HSV antibody i.p. 3.5 days after infection, n=10, c) 12% $CO_2$ for 1.5 hours, 3 times per day, n=10, and d) both antibody and $CO_2$, n=10. Illness rates were assessed twice daily. In addition, two groups of 5 mice each were studied for the effect of $CO_2$ on the BBB to protein. Albumin was conjugated with Na-Flourescein and injected via a tail vein 3.5 days after HSV innoculation in both groups. In the first group, CSF and blood were obtained 2 hours later. The second group was treated with 12% $CO_2$ for 1.5 hours and CSF and blood obtained after a 2 hour circulation time.

Illness rates can be seen in Table 1. $CO_2$ reduced the illness rates. Prior treatment with antibody did not further reduce this rate. Flourescence spectrophotometric analysis of the matched CSF and blood samples indicated that $CO_2$ increased the CSF/plasma ratio by 23%.

These preliminary results indicate that high dose $CO_2$ reduced the illness rates in this mouse model of HSV myelitis. Since the presence of high titer antibody did not potentiate $CO_2$, the positive effect of $CO_2$ may not be due to increased brain levels of antibody, but may be due to either: a) increased brain levels of other immune substances, or b) direct effects of $CO_2$ on immune function or virus survival.

Table 1. Number Ill divided by Number Inoculated

| Group | No $CO_2$ | $CO_2$ |
|---|---|---|
| HSV | 6/10 | 1/10 |
| HSV + Anti HSV Ab | 5/10 | 3/10 |
| TOTALS | 11/20 | 4/20[a] |

(a) $P<.05$ vs Non-$CO_2$ treated, chi-square

270.4 LIPID CONTENT OF SWINE INFLUENZA AND OTHER VACCINES. S.W. Brostoff*, E.L. Hogan, S. Dasgupta*, J.-L. Chien*, R.E. Erwin* and K.C. Leskawa* (SPON: J. Zemp). Department of Neurology, The Medical University of South Carolina, Charleston, SC 29425

An extensive immunization program conducted in the United States in 1976 to reduce the threat of an influenza epidemic caused by a swine flu strain is reported to have been associated with an increased incidence of Guillain-Barre syndrome (GBS). To search for a causative agent, experimental allergic neuritis (EAN) has been used as an animal model, since it shares many features of GBS. There have been many studies on the roles of lipids, either alone or in combination with protein, in causing experimental demyelinating diseases. Among these are galactosylcerebroside (GalCer), gangliosides and phosphatidylserine (PS). A qualitative and quantitative study was undertaken in order to assess whether a difference in the presence of these and other lipids could be associated in a meaningful way with swine flu vaccines. Six lots of swine flu vaccines (from different manufacturers) were compared to other influenza vaccines and non-influenza vaccines (Hepatitis; Rubella; and Diphtheria, Tetanus and Purtussis).

Cholesterol content varied from 3.0 to 44.0 ug/ml vaccine, but no major differences were found between the different types of vaccines. Cholesterol esters were not detected in any of the samples, and the non-influenza vaccines had a higher content of monoglycerides.

Total phospholipid content varied from 0.012 to 0.101 umole Pi/ml vaccine, and again major differences between types were not observed. Influenza vaccines roughly paralleled normal human plasma, in terms of individual phospholipids, with phosphatidylcholine, sphingomyelin and phosphatidic acid being the major phospholipids. Appreciable amounts of phosphatidylethanolamine were found in only one swine flu vaccine. PS was not detected in any swine flu vaccines, but was present in other flu vaccines, suggesting that PS-protein complexes do not play a role in the occurence of GBS.

Similarly, this study does not support the concept that gangliosides are involved in eliciting GBS, since only two of the six swine flu vaccines showed trace amounts of ganglioside (less than 0.5 ug/ml vaccine) and all three of the non-swine flu vaccines contained ganglioside (GM3 in all cases). GalCer was found in substantial quantities in swine flu vaccines, and was absent or present in trace amounts in the non-influenza vaccines. GalCer was also found in the other influenza vaccines (non-swine flu). With the knowledge that anti-GalCer serum causes demyelination under experimental conditions, the presence of this compound in commercial vaccines does warrant further attention.

Supported in part by NIH grant NS11867.

270.5 MONOCLONAL ANTIBODIES AGAINST DIHYDROLIPOAMIDE REDUCTASE [NAD+] (LAD). E. Brownridge*, R.A.P. Kark, P. Weisz-Carrington*, and M. Beene*. From the Depts. of Neurology and Pathology, VAMC and LSUMC School of Medicine, Shreveport, LA 71130.

The mitochondrial enzyme dihydrolipoamide reductase [NAD+] (lipoamide dehydrogenase, LAD, EC 1.6.4.3) is a part of the pyruvate dehydrogenase complex. Abnormalities of LAD have been associated with several inherited diseases of the brain. It is questioned whether the enzyme changes are primary or secondary. Precise, specific antibodies could help to answer this question. To seek monoclonal anti-LAD antibodies, 5 mice were hyperimmunized with purified pig-heart LAD (Sigma). Four produced antisera. Spleen cells from two were fused with non-secreting murine myeloma cells (P3X63-AG8.653) using polyethylene glycol. About $3x10^5$ spleen-cells were placed in each of 576 culture wells and grown for two weeks in hypoxanthine-aminopterine-thymidine medium with murine peritoneal "feeder" cells. Aliquots of supernatants from each well were examined by enzyme-linked immunoabsorbance assay (ELISA) for anti-LAD activity in an assay with sequential layers of hybridoma supernatant, LAD, and rabbit polyclonal anti-LAD IgG (purified by immunoabsorbance on an LAD-Sepharose column) which was tagged with horse-radish peroxidase (HRP). The ELISA was specific for the injected purified LAD, which has been used by others to study the amino acid sequence and three-dimensional structure of the enzyme. Cells from positive wells were cloned by limiting dilution and grown in the presence of feeder cells. Production of monoclonal anti-LAD was assessed by ELISA with LAD, monoclonal hybridoma supernatant, and HRP-tagged anti-mouse IgG, a more sensitive but equally specific reaction. We isolated 23 clones. As these appear to differ in avidity for LAD, they may represent several distinct clones.

270.6 RNA VIRUS INFECTIONS OF MOUSE SENSORY NEURONS IN CULTURE. R.J.Ziegler and E.K.Stauffer Univ. of Minn. Medical School, Duluth, MN. 55812 and D.R.Carrigan Univ. of Maryland Medical School, Baltimore, MD. 21201

Several RNA viruses are known to be neurotrophic. Some of these viruses like rabies are cytolytic to neurons, while other viruses like subacute sclerosing panencephalitis (SSPE) virus are non-cytolytic to neurons. We exposed two different types of NIH Swiss mouse dorsal root ganglia (DRG) cell cultures to four RNA viruses in order to determine if non-cytolytic viral replication or viral antigen synthesis occured. Cultures displaying either of these phenomenon were examined for changes in evoked action potentials (EVAPs).

DRGs were removed from 13-day mouse fetuses and dissociated cultures of 1) purified sensory neurons or 2) glia and sensory neurons were prepared. One to two week old cultures were exposed to either mumps virus (Kilhim strain), measles virus (Edmonston strain), SSPE virus (Mantooth strain), or ecotrophic murine leukemia virus (Lake Casitis strain) and viral replication monitored at various times post-infection (pi) by standard plaque assay or the XC assay for the Lake Casitis virus. Cultures not producing infectious virus were examined for the synthesis of specific viral antigens by the indirect immuno-fluorescent technique.

Mumps virus actively replicated in the sensory neurons and caused changes in the waveform of EVAPs. Measles virus replicated at low levels in cultures infected for three days or three weeks, although the number of neurons synthesizing measles antigens increased from 1% to 50% during this time period. Some measles virus-induced changes in EVAPs were observed. SSPE virus did not replicate, produce viral antigens, or change EVAPs during a three week infection period. Neurons exposed to Lake Casitis virus gave ambiguous results. More virus was detected at later times pi, but viral envelope glycoprotein antigen was not detected in these purified neuron cultures. Similer cultures observed at three to four weeks pi contained many large multi-nucleated neurons which also contained no envelope antigen. Large numbers of glial cells which did contain antigen reappeared in the older cultures. EVAPs from the multinucleated neurons appeared normal. The multinucleated neurons may be caused by fusion from without due to the virus produced by the glial cells in the cultures. Futher investigations of the phenomenom are in progress.

270.7 EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS (EAE) IN RATS: SEVERITY OF PERIPHERAL NEURITIS CAUSED BY VARIOUS NEURAL ANTIGENS. A. M. Kelahan and M. J. Buckley*. Dept. of Neurology, Northwest. Univ. Sch. Med., Chicago, IL 60611.

EAE is an experimental form of inflammatory demyelination which results from the innoculation of a host organism, typically a rodent, with a neural antigen from a non-host species, along with an immune system potentiating adjuvant. Because the resultant pathology compares favorably to that known to occur in multiple sclerosis (MS), and because of similar immune system involvement, EAE has been regarded for years as a prime animal model of MS. However a commonly reported feature of clinical EAE in rats is peripheral neuritis, which is not known to occur in pure MS. The purpose of this study was to compare the degree of peripheral neuritis caused by the exposure of rats to one of several types of neural antigens.

The neural antigens utilized were: a) whole guinea pig spinal cord myelin basic protein (MBP); b) a synthetic encephalitogenic peptide (amino acid chain 68-84 of guinea pig spinal cord MBP); c) lymph node cells (LNC) isolated from rats previously affected with EAE and then cultured with MBP; and d) MBP-cultured LNC (as in c above) followed within 10 days by guinea pig spinal cord MBP. Sensitization was accomplished via intradermal innoculations of the hind foot pads (a and b), intravenous injections (c) or both routes of administration (d). At various times during acute clinical stages and recovery, compound motor action potentials (CAP) evoked by electrical stimulation were recorded from the caudal nerve-muscle complex. Recordings were made at normal tail temperature and during heating (14-15 $^0$C above normal) and cooling of the tail. The presence and degree of peripheral neuritis in EAE-diseased rats was defined by changes in CAP propagation velocity, amplitude and initial latency as compared to similar recordings in normal rats.

In all cases of EAE sensitised rats, significant differences in measured CAP parameters were observed only in those rats exhibiting clinical signs of motor deficits; rats which had recovered from clinical EAE were not different from normal. Of the four different neural antigen groups, rats innoculated with the synthetic peptide displayed the least involvement of peripheral nerves, as indicated by normal appearing CAPs. In the other neural antigen groups, amplitude was the most significantly affected CAP parameter: a) amplitudes at normal tail temperature tended to be reduced in size and b) amplitude decreased much faster during tail heating as compared to normal. In some rats the heat lability of CAP amplitude was so profound as to reach, and maintain, a zero level during most of the recording session; lowered blocking temperature for transmission of electrical activity has been previously defined as a hallmark indicator of peripheral nerve pathology. Other CAP parameters were only sporadically different from normal in EAE-diseased rats and were not considered as good of indicators of peripheral nerve condition as CAP amplitude.

The results of these studies demonstrate that a) EAE can occur as a relatively pure CNS disorder and b) a major encephalitogenic role of MBP may reside in a specific peptide chain of whole MBP.

270.8 THE EFFECTS OF STEROIDS AND NON-STEROIDAL ANTI-INFLAMMATORY AGENTS ON VASCULAR PERMEABILITY IN THE C6 SPHEROID IMPLANTATION GLIOMA MODEL. H. Reichman*, C. L. Farrell*, R. F. Del Maestro* (SPON: M. Rathbone). Brain Research Laboratory, Victoria Hospital, London, Ontario, N6A 4G5

Cerebral edema associated with intracranial tumours is a consequence of breakdown of the normal permeability conditions of the blood brain barrier (BBB). The intracerebral spheroid implantation technique is a good model for primary brain tumours in that it has many features that mimic the growth and behaviour of primary intracranial tumours in humans. Tumour growth and vascular permeability have been characterized in this model. Edema was localized using fluorescein-conjugated antibodies to serum albumin. We have used Evans Blue (EB) as a marker to quantitate the leakage of serum constituents from tumour vessels, and have also quantified the ultrastructural features associated with the BBB in vessels in and around the tumour. Steroid therapy with methylprednisolone and dexamethasone significantly decreased vessel permeability as has been demonstrated in other experimental models and in humans. Because these parameters have been defined and quantified, this is an excellent model for assessment of new clinical therapies on edema formation. There are complications associated with long term steroid therapy. We used this model to examine the effects of two non-steroidal anti-inflammatory agents, indomethacin and ibuprofen, on edema-associated EB leakage in this model. Indomethacin significantly reduced EB leakage whereas the effect of ibuprofen requires further investigation. This suggests that non-steroidal anti-inflammatory agents may be a possible alternative to steroid therapy of patients with brain tumours.

271.1 DETECTION OF A NEURITE PROMOTING FACTOR WITH SOME SIMILARITY TO NERVE GROWTH FACTOR IN CONDITIONED MEDIA FROM RAT PRIMARY SCHWANN CELLS AND ASTROGLIA. J. Assouline*, E. P. Bosch*, R. Lim, R. Jensen* and N. J. Pantazis. Depts. of Anatomy and Neurology, Univ. of Iowa Medical College, Iowa City, IA 52242.

For unexplained reasons, numerous types of cells in culture, synthesize nerve growth factor (NGF) and secrete it into their feeding medium (conditioned medium). We investigated the possibility that primary Schwann cells and astroglia cells synthesize NGF. Primary Schwann cell cultures were established from newborn rat sciatic nerve. Fibroblasts were eliminated by both cytosine arabinoside treatment and an immunoabsorption technique. Indirect immunofluorescence for RAN 1 (specific for Schwann cells) and Thy 1.1 (for fibroblasts) revealed that the cultures contained 95-99% Schwann cells. Astroglia cells were established from 18d fetal rat brain. Indirect immunofluorescence revealed >90% astroglia (positive for glial fibrillary acidic protein), 8% thy 1.1 positive fibroblasts and <2% galactocerebroside positive oligodendrocytes. To obtain conditioned medium (CM), the established cells were grown to confluence in serum containing medium and then were switched to a serum-free defined medium (N2). After 2-4d, the CM was collected and processed for study.

Four methods were used to detect NGF in the CM: 1. β-NGF specific radioimmunoassay (NGF-RIA) using an antibody to mouse β-NGF; 2. β-NGF radioreceptor assay (NGF-RRA) using the NGF receptor from rat pheochromocytoma cells (PC12); 3. Bioassay on chick embryo, dorsal root ganglia (DRG); 4. Bioassay on PC12 cells. The NGF-RIA detected low levels of β-NGF (14-68 pg/ml), whereas the NGF-RRA detected 10-25 fold more NGF. Since the receptor used in the NGF-RRA is derived from rat cells, this assay may be more specific for rat NGF than the NGF-RIA. In the DRG bioassay, extensive neurite outgrowth was observed for both Schwann cell and astroglia cell CM. Similar outgrowth was seen with these samples in the PC12 cell bioassay. Antibody to mouse β-NGF did not inhibit this outgrowth in either bioassay.

In conclusion, several results, including positive NGF bioassays and detection of NGF by RIA and RRA, suggest that NGF is present in both Schwann and astroglia cell CM. Since the Schwann and astroglia cells were derived from the rat, the inability of mouse β-NGF antibody to inhibit neurite outgrowth may be due to species differences in NGF. These cells may produce a novel neurite promoting factor(s) which shares the biological activity of NGF and binds to its receptor, but possesses different antigenic determinants. Further characterization is underway. (Supported by NIH grant GM28644 to N.J.P. and NSF grant BNS-8308341 and NCI grant CA31896 to R.L.).

271.2 NGF-LIKE IMMUNOREACTIVITY IN THE CENTRAL AND PERIPHERAL NERVOUS SYSTEM OF THE FETAL RAT. Å. Seiger,* C. Ayer-LeLievre,* T. Ebendal,*[1] and L. Olson* (SPON: Å. Seiger, Member ENA). Dept. of Histology, Karolinska Institute, Stockholm, Sweden, and [1]Dept. of Zoology, Uppsala Univ., Uppsala, Sweden.

The regional distribution of nerve growth factor-like immunoreactivity is described in 15-17-day rat fetuses based on immunohistochemistry using affinity-purified polyclonal antibodies against mouse submaxillary gland nerve growth factor (NGF). In CNS, immunoreactivity was seen along neural pathways, such as dorsal and ventral fascicles in the spinal cord, fascicles in formatio reticularis, commissura posterior, fasciculus retroflexus and stria terminalis. These fluorescent pathways did not show discernable individual immunoreactive nerve fibers. Positive receptor nerve cells were seen in the immature olfactory mucosa and, at fetal day 17, weakly fluorescent retinal ganglion cells were found. Afferent fiber bundles from these receptor cells contained strongly positive individual nerve fibers. Fiber bundles could be followed from retina through the optic stalk to the chiasm. Numerous strongly positive fiber fascicles were seen to converge from the olfactory mucosa and enter the olfactory bulb ventromedially. Along the dorsal surface of cerebral cortex and tectum from 15-day fetuses a system of fibrous structures appeared neuronal and varicose on longitudinal sections.

In cerebral cortex of 17-day fetuses, the superficial zone was negative, whereas material in the intermediate zone interspersed between the differentiating neurons and growing axons was strongly positive. In many peripheral sensory and autonomic ganglia the neuronal perikarya were negative but surrounded by fascicles of NGF-positive fibers. Both proximal and distal roots were strongly positive as were peripheral branches of nerve bundles in many areas, including dermis, tongue and salivary glands. We conclude that immunohistochemistry using affinity purified antibodies to NGF reveals a widespread distribution of a NGF-like material in many areas of the central and peripheral nervous system of the prenatal rat. The specificity and implications of the observations for brain growth will be discussed, particularly in relation to recent reports about the presence of messenger RNA for NGF in the mammalian brain.

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271.3 SYNTHESIS OF A NERVE GROWTH FACTOR-LIKE MOLECULE BY RAT PHEOCHROMOCYTOMA CELLS IN CULTURE. I. Kim* and N.J. Pantazis. Dept. of Anatomy, Univ. of Iowa Medical College, Iowa City, IA 52242.

The PC12 cell line, cloned from a rat adrenal pheochromocytoma (Greene, L.A. and Tischler, A.S., Proc. Natl. Acad. Sci. USA, 73:2424, 1976), has been used to study the mechanism of action of nerve growth factor, NGF. In the presence of NGF, the PC12 cells will extend processes (neurites) similar to those seen when embryonic sensory and sympathetic neurons are cultured with NGF. Unlike embryonic sensory and sympathetic neurons, it is thought that the PC12 cells do not require NGF for survival. However, various types of cells in culture, including fibroblast, muscle and glia, synthesize NGF and secrete it, thereby conditioning their feeding medium. We investigated the possibility that PC12 cells have NGF in their conditioned medium.

PC12 cells, a gift from Dr. Lloyd Greene, were initially grown in RPMI-medium containing 5% fetal calf serum and 10% horse serum. In these conditions, the great majority of cells did not attach to the flask but grew in suspension. With time, increasing numbers of cells attached. At this point, the cells were divided into floating cells (PC12F) and attached cells (PC12A). To obtain conditioned media (CM), both PC12F and PC12A cells were grown to high density with serum containing medium and then were switched to a serum-free defined medium (N2).

Three methods were used to test for NGF in the concentrated CM from PC12F and PC12A cells: (1) β-NGF specific radioimmunoassay (NGF-RIA) using an antibody prepared against mouse β-NGF; (2) β-NGF radioreceptor assay (NGF-RRA) using NGF receptors on PC12F cells; (3) NGF bioassay using the PC12F cells which respond to NGF. Both NGF-RIA and NGF-RRA detected an NGF-like molecule in the concentrated CM from both the PC12F and PC12A cells. When the samples were tested in the NGF biological assay, the PC12A CM stimulated extensive fiber outgrowth, whereas the PC12F CM produced marginal fiber outgrowth. Polyclonal antibody against mouse β-NGF did not inhibit neurite outgrowth.

In conclusion, NGF-RIA and NGF-RRA suggest that an NGF-like molecule is present in PC12F and PC12A CM. This result is supported, in the case of PC12A CM, by NGF bioassay where a positive neurite outgrowth was observed. Anti-NGF antibody did not inhibit the fiber outgrowth. This may be due to species differences in NGF since the antibody was raised against mouse β-NGF while the PC12 cells are derived from rat. Further biochemical characterization of the NGF-like molecule produced by PC12 cells will determine its similarity to mouse NGF. There are numerous autocrine systems described for various growth factors. Whether or not this NGF-like molecule produced by PC12 cells can act on PC12 cells in an autocrine fashion is unknown. (Supported by NIH grant GM28644 to N.J.P.).

271.4 ENTORHINAL LESIONS RESULT IN INCREASED NEURITE-PROMOTING ACTIVITY IN MEDIUM CONDITIONED BY HIPPOCAMPAL SLICES. K.A. Crutcher and F. Collins, Dept. of Anatomy, University of Utah School of Medicine, Salt Lake City, Utah 84132.

Medial septal lesions result in increased levels of NGF-like activity in medium conditioned by exposure to hippocampal slices (Collins and Crutcher, this volume). In order to determine whether removal of other hippocampal afferent fibers would also affect such neurite-promoting activity we made unilateral electrolytic lesions of the entorhinal cortex in four adult female albino rats and unilateral injections of kainic acid (.4ug) into the hippocampal formation of four additional rats. Entorhinal lesions have been shown to result in extensive denervation of the ipsilateral dentate granule cells. Kainic acid injections result in destruction of hippocampal pyramidal cells with subsequent degeneration of contralateral (commissural) inputs to the granule cells. Slices (dentate-CA3) were prepared from the ipsilateral (entorhinal animals) and contralateral (kainic acid animals) hippocampal formation one week after the lesion and placed in culture medium for two days. Slices from control (unlesioned) animals were treated in the same way. The conditioned medium was then assayed for its effect on neurite outgrowth from explanted 9-day old chick lumbar sympathetic ganglia.

Entorhinal lesions resulted in increased neurite-promoting activity of the medium conditioned by hippocampal slices ipsilateral to the lesion when compared to medium conditioned by control slices or by slices contralateral to kainic acid injections. Preliminary results indicate that the increased neurite-promoting activity following entorhinal lesions is blocked by antibodies to NGF. These results suggest that removal of the entorhinal, but not the commissural, input to the dentate granule cells results in increased neurite-promoting activity in medium conditioned by exposure to slices of the dentate gyrus-CA3 region. A similar increase in growth activity is obtained following medial septal lesions and has been proposed to correlate with the sympathetic sprouting response observed in vivo (Collins and Crutcher, this volume). Entorhinal lesions do not result in sympathetic sprouting but do elicit proliferation of septohippocampal terminals. Commissural denervation does not evoke sprouting of either system. As a working hypothesis, we propose that the increased growth activity observed following entorhinal lesions correlates with the septo-hippocampal sprouting response. If the activity turns out to be related to NGF, this would be consistent with results obtained by other investigators demonstrating effects of NGF on septohippocampal neurons. Supported by NIH grant #NS17131.

271.5 NGF-LIKE ACTIVITY IN THE RAT HIPPOCAMPAL FORMATION: REGIONAL DISTRIBUTION AND INCREASE AFTER MEDIAL SEPTAL LESION. F. Collins and K. A. Crutcher Dept. of Anatomy, Univ. of Utah Sch. of Med., Salt Lake City, UT 84132

Sympathetic axons from nearby blood vessels grow into the hippocampal formation following a medial septal lesion that destroys the septal input to the hippocampal formation. This invasion of sympathetic axons is analogous to that observed when nerve growth factor (NGF) is injected directly into regions of the CNS. This analogy suggested that sympathetic ingrowth may result from increased NGF-like activity in the hippocampal formation after a medial septal lesion. Sympathetic ingrowth is densest in the dentate gyrus-CA3 region of the hippocampal formation, with little or no growth into the CA1 region. One would, therefore, expect the dentate-CA3 region to have the highest concentration of the putative NGF-like activity. Our experimental results support the hypothesis that there is NGF-like activity in the rat hippocampal formation, whose distribution and concentration is correlated with sympathetic sprouting after septal denervation.

Conditioned medium, prepared from slices of the dentate-CA3 and CA1 regions of the hippocampal formation, was assayed for its ability to increase neurite growth from embryonic chick sympathetic ganglia in culture. Some rats were given prior denervating medial septal, or various nondenervating control, lesions. Conditioned medium from the dentate-CA3 region contained approximately 2 fold more activity than from the CA1 region. Activity in conditioned medium from both regions of the hippocampal formation increased 1.5-2 fold within 1 week after medial septal, but not control, lesions. In conditioned medium from the dentate-CA3 region, this elevation in activity was observed even when the medial septal lesion had been given as long as 24 weeks earlier. In addition, the neurite stimulating activity in hippocampal conditioned medium was blocked by a specific, affinity purified antibody to mouse submaxillary gland NGF. These results indicate that: 1) Conditioned medium from the adult rat hippocampal formation contains an activity which is able to stimulate sympathetic neurite growth and is antigenically related to NGF. 2) The increase in this activity after a medial septal lesion and its differential distribution within the hippocampal formation correspond to the induction and pattern of sympathetic sprouting, respectively. Experiments directed at understanding the effect of other denervating lesions are described in a companion abstract (Crutcher and Collins, this volume). Supported by NIH Grant #NS 17131.

271.6 GLIAL FACTORS IN THE BRAIN. T. Amano. Dept. of Neuroscience, Lab. of Neurochemistry, Mitsubishi-Kasei Institute of Life Sciences, 11 Minamiooya, Machida-shi, Tokyo 194, Japan

Monoclonal antibodies to 2.5 S nerve growth factor (swiss webster) were produced by in vitro immunization in which BALB/C mouse spleen cells were fused with P3U1 myeloma cells. Three kinds of monoclonal antibodies were obtained. 1) Monoclonal antibodies which recognize antigenic determinants in astrocytes in the mouse and rat brain. 2) Monoclonal antibodies which recognize antigenic sites in neurons. 3) Monoclonal antibodies which had no localization in the brain. The first two groups had no biological activities which inhibit neurite outgrowth of spinal ganglion of 8-day-chick embryo. The third group had biological activities. Western blottings of extract of submaxillary glands of C57BL/6j mouse revealed exactly the same localization bands on nitrocellulose with 38,000 and 60,000 in molecular weight as with polyclonal anti-NGF. In purified preparations of NGF from mouse submaxillary gland, immunoblottings revealed one band with 14,000 in molecular weight. The third group of hybridomas which had quite similar properties to polyclonal anti-NGF, revealed clear localization in astrocytes in the brain after the stab wound in the cortex. In other experiments using secreted proteins from C6Bu1 rat glioma cells in serum-free culture for 48 hours as antigens, one of the hybridomas recognized both astrocytes and neurons in the brain. Western blottings showed quite similar pattern to monoclonal antibodies to NGF in two dimensional electrophoresis. Together with these results, we conclude that neurotrophic factor in the brain may exist in astrocytes and may be related to nerve growth factor.

271.7 **SYNERGISM BETWEEN NGF AND LAMININ IN PROMOTING SURVIVAL AND NEURITE OUTGROWTH FROM PURIFIED SENSORY NEURONS IN LOW DENSITY CULTURE.** A.I. Millaruelo*, M. Nieto-Sampedro and C.W. Cotman. Department of Psychobiology, University of California, Irvine, CA 92717.

Nerve growth factor (NGF) is known to promote survival and neurite outgrowth from explanted sensory and sympathetic ganglia in culture[1]. However, in the absence of non-neuronal cells a relatively small percentage of sensory or sympathetic neurons survive in the presence of NGF[2,3], and very few of these cells exhibit neurite outgrowth. The survival and outgrowth of neurites from sympathetic neurons in response to NGF is potentiated by the basement membrane protein laminin[3]. The present work evaluates the role of laminin, NGF and extracts of normal and injured rat brain in the survival and outgrowth of neurites from sensory neurons.

Chick embryo dorsal root ganglion (DRG) neurons, freed (90%) from non-neuronal cells, were cultured at low density (3500 cells/cm$^2$) in medium containing 10% fetal calf serum on a polylysine substrate. Under these conditions, only 5% survived for 48 h, and none put out neurites (determined at 4 h and 48 h). If the medium was supplemented with NGF (up to 50 μg/ml), survival increased to a maximum of 33% of the neurons seeded but only 18% of them bore neurites. Treatment of the growth substrate with laminin (10 μg/ml, 2 h) by itself did not increase survival, although the few surviving neurons had neurites at 48 h. The simultaneous presence of NGF and laminin dramatically enhanced (to 100% of the neurons seeded) both survival and neurite initiation and outgrowth of purified DRG neurons. Thus, endogenous laminin secreted by non-neuronal cells may play a role not only in the enhancement of neurite outgrowth from sensory neurons in response to NGF[4], but also in the survival of these neurons.

NGF and laminin also acted synergistically to promote neurite outgrowth from purified ciliary ganglion neurons. However, survival of these cells seemed to require additional neurotrophic support.

Extracts of normal and injured adult rat brain contained NGF-like survival-promoting and neurite-promoting factors for DRG neurons. The percentage of neurons with neurites increased from 31% to 75% when cultured on laminin-treated vs. untreated polylysine.

Our work highlights the importance of synergistic trophic interactions for both neuronal survival and differentiation, and emphasizes the necessity of using pure cell populations to analyze individual effects of growth factors.

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271.8 INSULIN ATTENUATES NEUROTOXIC EFFECTS OF TAXOL ON VENTRAL REGIONS OF FETAL MOUSE SPINAL CORD EXPLANTS INDEPENDENT OF NGF-RESCUE OF ATTACHED DORSAL ROOT GANGLION (DRG) NEURONS. E.R. Peterson* and S.M. Crain (SPON: E.B. Masurovsky). Dept. of Neuroscience, Albert Einstein College of Medicine, Yeshiva University, Bronx, N.Y. 10461

After exposure of fetal mouse cord-DRG explants to the plant anti-tumor alkaloid, taxol, DRG neurons remain dependent on high levels of exogenous NGF for survival, long after drug withdrawal (Peterson & Crain, Science 217, 377, '82). While neurons in dorsal cord explants do not normally require a DRG input for longterm maintenance, during and after taxol exposure these cells also become more dependent on trophic factors provided by NGF-enhanced DRG neurite projections. Ventral cord neurons in these same taxol-treated explants show extensive degeneration.

In the present study, mouse cord-DRG explants (E-13) with attached DRGs were cultured in control media containing NGF (300 units/ml) for 4 days, and then exposed to taxol (1-2 um) ± insulin (20 ug/ml, bovine pancreas, Sigma) + NGF for 4 days. After taxol withdrawal, the cultures were maintained in control media with NGF ± insulin for 1-2 wks. Increased numbers of ventral cord neurons survived with insulin supplement, while in control cultures, the ventral cord tissue was more often reduced to a monolayer of glial cells. Insulin markedly attenuated the usual degeneration of taxol-treated ventral cord neurons, even when the NGF level was transiently lowered after drug withdrawal to levels that resulted in severe damage to the DRG and dorsal cord neurons. Electrophysiologic tests showed that characteristic synaptic network discharges could be generated in the ventral cord tissue "rescued" by insulin.

While development of many ventral cord neurons is essentially normal in longterm explant cultures without addition of insulin, following exposure to taxol they become dependent on insulin (or insulin-like factors), just as DRG neurons remain dependent on NGF and dorsal cord neurons become dependent on DRG neurite inputs. In the absence of these enhanced extrinsic influences, taxol exposure results in severe cytotoxic abnormalities in DRG and cord neurons. In drug-free cultures, DRG and cord neurons probably survive by utilizing small amounts of essential trophic factors present in serum/embryo-extract nutrient media or provided by supporting cells. Perhaps certain types of neurologic degenerative disorders might involve defective regulation of a putative endogenous factor with activity similar to that of taxol (Schiff et al, Nature 277, 665, '79) in specific groups of neurons (see rev. by Crain & Peterson, in Cellular & Molecular Biology of Neuronal Development, ed. I. Black, Plenum '84). (Supported by grants NS-19611 to Dr. P.S. Spencer and BNS-821847 to S.M.C.)

271.9 **LAMININ AND PYRUVATE TOGETHER SUPPORT SHORT TERM SURVIVAL OF RAT SEPTAL NEURONS IN LOW DENSITY, SERUM-FREE CULTURES.** S. K. R. Pixley and C. W. Cotman, Department of Psychobiology, U. of Cal., Irvine, CA 92717.

Laminin has neurite-promoting effects for a variety of cultured neurons and has been implicated in neurite-promotion during nervous system development and injury repair. We report here that laminin is also capable of supporting survival of central neurons maintained in culture. The survival effect is not observed in the absence of pyruvate, a trophic component of astrocyte conditioned media.

Septal neurons from embryonic day 18 rats did not survive for 24 hours when dissociated and cultured at low density in a standard serum-free medium containing the N1 supplements. However, if both laminin and pyruvate were present in the culture system, significant 24 hour cell survival was observed. Laminin supported cell survival only if it was present in the culture system during cell plating (either attached to the substrate or soluble in the medium); laminin did not support cell survival if it was added after cells had adhered to polylysine-coated culture wells. Variations in laminin concentration did not affect cell attachment or the percent of total cells that were identified as neurons. Laminin-supported neurons survived for 4 days in culture, but the cell numbers were 53% of the 24 hour values.

Laminin also acted as a neurite-promoting factor in these cultures. Significant numbers of septal neurons were capable of producing neurites, even at the lowest laminin concentrations tested. Above this baseline, the percent of neurite-bearing cells was proportional to laminin concentration. The rate of neurite initiation was also proportional to laminin concentration.

Thus, laminin promoted neuronal survival and sprouting if it was present during cell plating and if the cells had metabolic support in the form of pyruvate. No other trophic agents were required for survival.

Both neuronal process formation and neuronal death occur during development and after injury to the CNS. A variety of neurotrophic factors are present at both times and laminin may be one of them, acting as a survival promoting agent in vivo as it does in embryonic neuronal cultures.

Supported by grant MH19691 and NIH Fellowship NS07238.

271.10 GROWTH FACTORS, cAMP AND ELEVATED $K^+$ PREVENT THE DEATH OF CLONAL PC12 PHEOCHROMOCYTOMA CELLS IN SERUM-FREE MEDIUM. R.E. Rydel and L.A. Greene*, Department of Pharmacology, New York University School of Medicine, New York, NY 10016

In serum-containing medium (85% RPMI 1640, 5% fetal calf serum, 10% horse serum, and no nerve growth factor), cultures of PC12 cells show excellent viability and growth. When serum is withdrawn, 90% of the cells die within 4-6 days and 99% by 2-3 weeks. If nerve growth factor (NGF) is added at the time of serum withdrawal, the cells remain viable for at least 1 month and extend neurites (Greene, L.A., J. Cell Biol. 78:747, 1978). In the present study, we sought to determine a) if other conditions could mimic the survival properties of NGF in serum-free cultures of PC12 cells and, b) if so, whether these conditions produce other shared responses in PC12 cells.

Cultures of PC12 cells on collagen-coated dishes were washed three times with serum-free medium (RPMI 1640) and then re-fed with serum-free medium containing one of the agents listed below. Cultures were fed every other day and cell number per culture was determined after 1-3 weeks. 5 ng/ml fibroblast growth factor (FGF), 500 ng/ml insulin, 40mM $K^+$, 30μM forskolin, 0.5mM dibutyryl cAMP, and 1.0mM 8(4-chlorphenylthio)-cAMP all prevented death of PC12 cells in serum-free medium to a similar extent as that observed with 50 ng/ml NGF. Treatment with 5 ng/ml epidermal growth factor resulted in approximately half the number of surviving PC12 cells when compared with the above treatments. Dibutyryl cGMP (0.5-1.0mM), 1.0mM 8-bromo cGMP, 1.0mM sodium butyrate, phorbol myristate acetate (10nM and 1.0μM), vasoactive intestinal peptide (2.0-5000 ng/ml), arginine vasopressin (10-2500 ng/ml), 10μM dexamethasone, and platelet-derived growth factor (1 U/ml) did not promote survival of PC12 cells in serum-free medium. FGF produced stable neurites (> 3 cell diameters in length and containing growth-cone-like flattenings) after 3 days of treatment in serum-free medium. These processes appear quite distinct from those recently reported with FGF treatment of PC12 cells in serum-containing medium (Togari, et.al., J. Neurosci. 5:307, 1985). With the exception of NGF, none of the other agents caused neuritic outgrowth.

When added to serum-free cultures, all agents supporting survival shared the ability to yield rapid (< 1 hr) changes in the phosphorylation of specific cytoplasmic and nuclear proteins of PC12 cells, and induced cell flattening and spiking within 24 hours of addition. This suggests that phosphorylation may play a causative role in cellular survival and that the PC12 cell system may prove useful for studying mechanisms involved in neuronal cell death. Supported by NIH grant NS16036 and NIH predoctoral training grant GM07827.

271.11 ENHANCEMENT OF AChE ACTIVITY IN CULTURED SPINAL CORD NEURONS BY THYROTROPIN RELEASING HORMONE.
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Recent investigations have indicated that thyrotropin releasing hormone (TRH) displays neurotrophic properties with respect to motoneurons. The process of motoneuron cell death which occurs during embryonic development accounts for the loss of approximately 50% of the motoneurons in the lateral motor column of the chick between days 6 and 10. Weill (Neurosci. Abst. 10,641(1984)) found that TRH enhances spinal cord motoneuron survival when administered in vivo. Therefore, the current study was initiated to examine the effect of TRH on acetylcholinesterase (AChE, EC 3.1.1.7), an enzyme that is specifically associated with motoneurons in cultures of embryonic chick spinal cord.

The culture system employed day 6 embryonic chick spinal cords which were trypsinized and plated in collagen coated 16 mm multiwells at a density of 2 million cells/well in complete MEM containing 10uM cytosine arabinoside C. Cultures were fed every 2 days with complete MEM (MEM with 5% chick embryo extract, 10% horse serum, 2 mM glutamine, 50 U/ml penicillin, 50 ug/ml streptomycin and 2.5 mcg/ml fungizone). Cells were harvested at 2 day intervals over an 8 day period and extracts stored in liquid nitrogen until assayed. AChE, a marker for neuronal development, was assayed according to the method of Ellman using acetylthiocholine iodide as substrate.

The results show that AChE activity increased in control cultures two fold between days 1 and 8. Similar increases with culture age were observed in cultures treated with 50 nM TRH. In the TRH treated cultures, however, AChE activity was higher at all ages examined, with levels significantly elevated ($p<0.1$) by 45%, 86% and 44% at days 4, 6 and 8, respectively. Mean AChE values (mU/mg total protein ±SEM) for duplicate samples were: day 4 control, 4.97±0.67; day 4 TRH treated, 7.20±0.11; day 6 control, 8.24±0.06; day 6 TRH treated, 15.3±2.31; day 8 control, 8.97±1.16; day 8 TRH treated, 13.0±0.05.

Depolarizing conditions in general have been shown to be neurotrophic and we found that 25mM K+ also elevated AChE levels in cultured spinal cord neurons. Hence, it appears that the peptide hormone TRH has a similar trophic effect on spinal cord neurons in culture. Other enzyme markers and parameters will be explored to characterize the neurotrophic effect of TRH further. Supported by NIH grant NS18642.

271.12 TRANSFERRIN LEVELS IN AMYOTROPHIC LATERAL SCLEROSIS AND CONTROL SERA MEASURED BY RADIOIMMUNOASSAY. M.K.N. Patel and B.W. Festoff. Dept. Neurology, V.A. Medical Center, 4801 Linwood Blvd., Kansas City, MO 64128.

Previous research from our laboratory reported that peripheral nerve extract stimulated myogenesis in chick primary cells in culture (Popiela et al.; J. Neurosci. Res. 8:547-567, 1982), and also promoted long term survival and neurite outgrowth in cultured embryonic chick spinal cord neurons (Popiela et al. Cell. Mol. Neurobiol. 4:67-77, 1984). This trophic factor was identified as transferrin.

We asked the question, could transferrin also be involved in the etiology of amyotrophic lateral sclerosis? We chose to measure serum transferrin because the pathology involved peripheral motor neurons and muscle and blood components have access to both these tissues.

Human transferrin was labeled with Iodine-125 and used in a competitive binding assay with rabbit anti-human transferrin antibodies. The antigen-antibody removal by centrifugation was enhanced by preincubation with goat anti-rabbit IgG. The pellet was counted in a gamma counter. Our results shown in Table I below indicates that statistically there is no significant difference in serum transferrin levels in ALS and controls.

TABLE I

| SOURCE | NUM | MEAN | STD | STE |
|---|---|---|---|---|
| Group #1 (Controls) | 13 | 324.9231 | 89.6981 | 24.8778 |
| Group #2 (ALS) | 18 | 270.8889 | 96.1353 | 22.6593 |

F-TEST FOR EQUALITY OF POPULATION VARIANCES
F = 1.1487 DF = 17 & 12 P = 0.41138

| | | | | |
|---|---|---|---|---|
| Equal | (P > 0.05) | T = 1.5873 | DF = 29 | P = 0.12318 |
| Unequal | (P < 0.05) | T = 1.6058 | DF = 29 | P = 0.11916 |

Supported by grant from the Keith Worthing ALS Regional Research Center.

271.13 INFLUENCE OF POLYPEPTIDE GROWTH FACTORS ON ACETYLCHOLINESTERASE (AChE) AND AChE MOLECULAR FRACTIONS IN ANNEURALLY CULTURED ADULT HUMAN MUSCLE

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In skeletal muscle, AChE and its 16S endplate-specific molecular fraction are under neuronal influence. We have previously reported that fibroblast growth factor (FGF), epidermal growth factor (EGF), and insulin (I) increase the total number of acetylcholine receptors (AChRs) and AChR aggregation in aneurally cultured adult human muscle (Askanas et al, Neurol, 35:102, 1985). In this regard, the influence of polypeptide growth factors (PGFs) on cultured human muscle is very similar to the influence of soluble neuronal factors on cultured animal muscle. In the present study, we examined the influence of PGFs on AChE activity and AChE molecular fractions in cultured adult human muscle. Muscle cultures were established in 5 experiments, each from a different muscle biopsy. Cultures were initiated from $5x10^5$ cells/dish in 60 mm petri dishes and grown for 3 wks. In each experiment, 10 dishes were treated with combined addition of 50 ng FGF + 10 ng EGF + 10 ug insulin and 10 dishes received control medium (CM). AChE was extracted in buffer containing 1M NaCl,1% Triton X-100, and 1 mM EGTA in 10 mM Tris HCl pH 7.2. AChE was determined at 37°C by the method of Ellman et al (Biochem Pharm. 7:88, 1961) and expressed in optical density variation units 1hr at 412 nm lh. One optical density variation unit (1OD) corresponds to hydrolysis of 73.5 umol acetylthiocholine iodide, (Rieger and Vigny, J Neurochem 42:601,1984). AChE molecular fractions were characterized by sedimentation coefficients in a 5-20% sucrose gradient, using B-galactosidase as a 16S marker and alkaline phosphatase as a 6S marker. 200 ul samples were added to the gradient, which was subsequently centrifuged at 168,000 g for 16 hrs. In cultures treated with PGFs, total AChE activity was increased to 340% of the control value ($p=0.005$). However, there was virtually no difference in distribution of AChE molecular fractions between PGF-treated and control cultures. Control cultures had 62% 4S, 26% 10S, and 12% 16S, and PGF-treated cultures had 61% 4S, 28% 10S, and 11% 16S.

Our studies demonstrate: 1) the presence of end-plate-specific 16S molecular fraction of AChE in aneurally cultured human muscle; 2) significant influence of FGF+EGF+I on total AChE activity of cultured adult human muscle, without a preferential influence on the 16S molecular fraction, and 3) an action of PGFs similar to that of soluble neuronal factors, since the latter also increase total AChE without preferentially influencing the end-plate specific molecular fraction in chick cultured muscle (Siegel, Trends in Pharm. Sci. 4:131, 1983). Even though PGFs and soluble neuronal factors increase the total number of AChRs, AChR aggregation and AChE in cultured muscle, other factors and/or nerve-muscle physical contact may be necessary for preferentially influencing the 16S end-plate specific molecular fraction of AChE. (Supported by Muscular Dystrophy Association)

271.14 SERUM FREE, HORMONALLY-CHEMICALLY DEFINED MEDIUM FOR PRIMARY CULTURE OF HUMAN MUSCLE.

Valerie Askanas, Susan Cave, and W. King Engel.
Usc Neuromuscular Center, Los Angeles, CA, 90020

We have previously reported that addition of fibroblast growth factor (FGF), epidermal growth factor (EGF) and insulin to culture-medium containing 10% fetal calf serum (FCS) caused synergistic increase of creatine kinase and increase of total acetylcholine receptors (AChRs) and clustering of AChRs in cultured human muscle as compared to muscle cultured in non-supplemented medium (Ann. Neurol. 16:143, 1984, Neurol. 35:102, 1985). We now report that supplementation of medium with those three polypeptide growth factors and with several other components allowed establishment of a serum-free medium (SFM) in which development and maturation of adult human muscle are much better than in control medium (CM) containing10% serum. SFM is based on F14 medium (Proc.Natl. Acad. Sci. 69; 3180,1972) supplemented per 1ml with: 50 ng FGF, 10 ng EGF, 10 ug insulin, 50 ug transferrin, 10 nM dihydrotestosterone, 50 uM hydrocortisone, 5 nM estradiol, 25 pM triiodothyronine, 50 uM putrescine, 2x $10^{-4}$ units alpha-tocopherol, 1 uM leupeptine, 0.1 mM L-carnitine, 2.5 mg BSA, 15 mM (pH 7.4) Hepes buffer. Cultures were established on collagen-coated 35 mm petri dishes from trypsinized cells ($4X10^5$ cells per dish), in medium supplemented with 10% FCS + FGF+ EGF + insulin. After 24 hours, when the majority of cells were attached, this medium was replaced by SFM.

In five experiments, each from a different biopsy (total of 50 cultures), human muscle cultured for 4 weeks in SFM had 223% ($p<0.02$) increase of CK activity and 217% increase ($p<0.02$) of total AChRs compared to muscle cultured in CM. Isozymes of CK indicative of muscle maturation revealed 30% increase ($p<0.005$) of muscle (MM) band in SFM cultured muscle compared to control muscle. AChR clusters were increased 1122% ($p < 0.001$) in SFM cultured muscle compared to control muscle. In addition, spontaneous contractions were often observed in SFM-cultured muscle and very rarely in control muscle.

In summary: 1) we present the first demonstration that adult human muscle can be cultured in SFM; 2) SFM supports human muscle growth and maturation significantly better than serum-supplemented medium; 3) SFM provides an important environment in which one can selectively study the influence of various hormones and growth factors on human muscle carrying an abnormal gene.

(Supported by Muscular Dystrophy Association)

271.15 IN VIVO EFFECT OF A NERVE EXTRACT UPON NUMBERS OF ACETYLCHOLINE RECEPTORS IN DENERVATED SKELETAL MUSCLES.

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The appearance of new extrajunctional (exj) acetylcholine receptors (AChR) is a characteristic response of adult skeletal muscle to denervation. Although this has been shown to be due to an increase in messenger RNA's coding for AChR protein, the neural signal leading to the increased transcription of the AChR gene is not known. Apparently, however, some neural influence normally represses the synthesis of exj AChR. The objective of this study was to determine whether continual infusions of a nerve extract would retard the appearance of new AChR.

Immediately after bilateral denervation of soleus muscles in the rat hindlimb by sciatotomy, an extract of sciatic nerves (100,000xg supernatant of nerve homogenate) obtained from other rats was injected intramuscularly (20 µl of 2.0-7.1 mg protein/ml). Then mini-osmotic pumps (Alza Corp.) loaded with the same extract were implanted to release the extract directly onto the soleus muscle at a rate of 1 µl/hr for 66 hr. Contralateral solei received injections and infusions of Ringer's solution. Also, injections and infusions of liver extract (7.5 mg protein/ml) or purified transferrin (5.0 mg/ml) were performed. After denervation and infusion periods of 66 hr, the solei were dissected and divided into junctional (j) and exj regions. Triton X-100 solubilized AChR were extracted from homogenates of these muscle sections and incubated with 125I-alpha bungarotoxin (BUTX). Radioiodinated AchR were separated by Sephacryl S-200 chromatography and quantitated.

Based upon the binding of 125I-BUTX the number of j and exj AChR in the Ringer-infused denervated muscles was increased 4-5 fold. This is comparable to AChR levels observed in 66 hr denervated solei receiving no infusions. The AChR numbers in denervated solei receiving a high concentration nerve extract infusion (6.0-7.1 mg protein/ml) were reduced 58-71% and 24-57% in the j and exj regions, respectively. Infusions of transferrin or liver extract reduced the denervation induced increase of AChR <30%.

Our results show that there is an active component present in nerve extracts which prevents the increase in numbers of AChR which usually occurs after denervation. This activity is apparently present at a lower level in the non-neural liver extract. Transferrin, a known growth promoter, had only a small effect on muscle AChR number even at high concentrations. These results are consistent with the current hypothesis that axonally transported neurotrophic substances influence the synthesis of muscle AChR.

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272.1 CHARACTERIZATION OF NERVE GROWTH FACTOR (NGF) BINDING BY CULTURED NEURAL CREST CELLS. P. Bernd. Department of Anatomy, Mount Sinai School of Medicine of the City University of New York, New York, N.Y. 10029.

We have previously demonstrated by light microscopic radioautographic techniques that a subpopulation of cultured neural crest cells undergoing differentiation have specific NGF receptors. These cells are the likely targets of NGF during differentiation and development. This study was done to pharmacologically characterize the binding of NGF to neural crest cells (7 to 14 days past explantation). Neural crest cultures were prepared from the trunk region of 48 hr quail embryos, and subcultured after 3 to 5 days onto gelatin coated 100mm plastic Petri dishes (approximately 2 x $10^6$ cells/dish) or 16mm plastic wells (approximately 1 x $10^5$ cells/well). Cells were washed with chick embryo extract-free culture medium for 2 hr, and triturated off 100mm dishes prior to experimentation (HBSS without $Ca^{++}$ and $Mg^{++}$ and with 1mM EDTA). Neural crest cells in suspension were then exposed to varying concentrations of $^{125}$I-NGF (0.07 to 200 ng/ml, 130 cpm/pg, 1 hr, 37°C), after which 100ul aliquots were removed (approximately 100,000 cells/aliquot), layered over a 175ul cushion of 0.15M sucrose and spun (1 min, Beckman microfuge B). The tubes were then quickly frozen and the tips, containing the cell pellet, cut off. Both cell pellet and supernatant were counted in a Gamma counter. Controls included an excess of nonradioactive NGF (5 ug/ml), as well as $^{125}$I-NGF. The data indicate that $^{125}$I-NGF binding was saturable with less than 20% nonspecific binding. Scatchard analysis revealed the presence of one type (class) of receptors with a $k_d$ similar to that of the low affinity NGF binding site (approximately 3.2 nM). This was corroborated by displacement experiments ($k_d$ approximately 1.9 nM) in which cells were exposed to $^{125}$I-NGF (5 ng/ml), as well as varying concentrations of nonradioactive NGF (0 to 5 ug/ml) for 1 hr (37°C). Binding of $^{125}$I-NGF was sensitive to trypsin; only 25% specific binding remained following sequential exposure to trypsin (0.05%, 90 min) and $^{125}$I-NGF (5 ng/ml; 1 hr). Quantitation of surface-bound vs. internalized $^{125}$I-NGF by differential release of the former at low pH, high salt (0.2M acetic acid, 0.5M NaCl) revealed that 13% of the $^{125}$I-NGF was internalized following incubation for 1 hr. In addition, specific binding was determined to be about 175 cpm/ug protein. These findings indicate that differentiating neural crest cells differ in their $^{125}$I-NGF binding properties from embryonic chick sympathetic or dorsal root ganglia, known NGF targets, in that they lack a high affinity $^{125}$I-NGF binding site. This suggests that these cells, which have not terminally differentiated (i.e. cell division still continues), may express an early developmental form of the NGF receptor. Supported by grants from the NIH (#HD 17262) and the Dysautonomia Foundation.

272.2 DEVELOPMENTAL EXPRESSION OF NERVE GROWTH FACTOR RECEPTORS IN PRIMARY CULTURES OF NEURAL CREST CELLS. C.A.M. Greiner*, A.T. Lloyd*, and G. Guroff. Department of Neurology and Committee on Neurobiology, U. Chicago; Section on Growth Factors, National Institute of Child Health and Human Development, NIH, Bethesda, MD 20205.

It has been suggested that environmental factors may be involved in the phenotypic expression of neural crest cells; however, the nature and mechanism(s) of such epigenetic influences are unknown. Previous work from this laboratory (Dev. Brain Res., 7: 131, 1983) demonstrated that 5-day cultures of neural crest cells express receptors for nerve growth factor (NGF), a molecule necessary for the survival and development of various neural crest derivatives. The present study examines the expression of NGF receptors during the development of neural crest cells in vitro.

Neural crest primary cultures were established from the caudal 6-8 segments of 2-day quail embryos and maintained in α -MEM, 15% horse serum, and 10% chick embryo extract. Cultures aged 2-8 days were incubated with $^{125}$I-NGF for 1 hour at 37°C. Control cultures additionally received an excess of unlabeled NGF. NGF receptors were detected in cultures aged 4-8 days, although specific binding could occasionally be detected in 3-day cultures. The amount of binding, however, often varied considerably among cultures of the same age. Parallel cultures processed for indirect immunofluorescence revealed a subpopulation of neural crest cells that express NGF receptors. Large fluorescent aggregates were noted in 5- and 6-day cultures; similar smaller aggregates were observed in 4-day cultures. Some 3-day cultures exhibited faintly fluorescent cells; 2-day cultures were consistently nonfluorescent. Pigment cells did not appear to bind NGF.

In order to determine the magnitude of the NGF-binding population, 5-day cultures were incubated with fluorescein isothiocyanate-conjugated NGF for 24 hours at 37°C and analyzed cytofluorometrically. Preliminary results indicate that approximately 28% of neural crest cells in 5-day cultures express NGF receptors. Experiments are currently in progress using cell sorting techniques to isolate and characterize this neural crest subpopulation.

272.3 AN AUTORADIOGRAPHIC AND IMMUNOHISTOCHEMICAL DEMONSTRATION OF NERVE GROWTH FACTOR RECEPTOR-BINDING SITES IN RAT LUMBAR SPINAL CORD. E.M. Johnson, P.A. Lampe*, P.A.Osborne* and H.K.Yip. Dept. of Pharmacology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

The presence of nerve growth factor (NGF) receptors on terminal processes of dorsal root ganglion (DRG) neurons was inferred by the previous demonstration of the retrograde transport of $^{125}$I-NGF from the spinal cord to the DRG. The presence and distribution of NGF binding sites in the rat lumbar spinal cord was determined using light microscopic autoradiography and immunohistochemistry. A narrow band of high density of silver grains was observed in laminae I and II of the dorsal horn after injection into spinal cord of $^{125}$I-NGF or iodinated monoclonal antibody against NGF receptor. Injection of $^{125}$I-cytochrome c or coinjection of $^{125}$I-NGF with an excess amount of unlabelled NGF produced no specific binding. The immunohistochemical results with the monoclonal antibody were consistent with the autoradiographic data. Immunoreactive neuronal processes and terminals were also localized in the superficial laminae of the dorsal horn.

Dorsal rhizotomy resulted in a complete loss of NGF receptor-binding sites in the dorsal horn, as demonstrated by both autoradiography and immunohistochemistry, indicating that these receptors are contained in primary sensory afferents.

The distribution of NGF receptor-binding sites in the rat spinal cord was similar to that observed for substance P and other neuropeptides in the cord. Our results suggest that these peptidnergic neurons also contain NGF receptors at their terminals in the superficial layers of the dorsal horn. These findings are consistent with the idea that NGF derived from the CNS is important in the development and maintenance of sensory neurons. (Supported by NIH grant NS 18071).

272.4 COMPARATIVE DYNAMICS OF RETROGRADE TRANSPORT OF NERVE GROWTH FACTOR AND HORSERADISH PEROXIDASE IN RAT LUMBAR DORSAL ROOT GANGLIA. H.K. Yip and E.M. Johnson, Dept. of Pharmacology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

The dynamics of the retrograde transport of $^{125}$I-nerve growth factor (NGF) and horseradish peroxidase (HRP) in primary sensory neurons were studied in rat. After injection of $^{125}$I-NGF or HRP into crushed sciatic nerves, labelling was examined in spinal nerves, dorsal root ganglia (DRG), dorsal roots, and spinal cords. Retrograde transport of both $^{125}$I-NGF and HRP was first observed in DRG neurons 6 hr. after injection. This indicated that the rate of transport ( 7mm/hr) of these proteins was identical. Significant differences in the sizes of DRG neurons labelled by $^{125}$I-NGF were observed depending on survival time. No such difference was seen in HRP-injected animals at any given time. After HRP injection the total number of labelled neurons in the DRG was higher than after $^{125}$I-NGF injection. At six hours after the injection, 60% of all the HRP-labelled cells had a diameter greater than 25 μm; whereas 90% of all the $^{125}$I-NGF labelled cells had a diameter less than 25 μm. There was a gradual shift in the size of $^{125}$I-NGF labelled neurons towards larger diameter with longer survival times. Thus, 24 hr. after the injection 83% of the labelled cells had a diameter greater than 25 μm. The data suggest that small diameter neurons retrogradely transport and turnover NGF faster than larger diameter neurons. There was a preferential accumulation of silver grains in small DRG neurons (mean diameter 25 μm) at early survival times (4 and 8 hr.); an inverse situation (mean diameter 42 μm) occurred at late survival time (24 hr.). In contrast, the mean diameter of HRP-labelled neurons remained constant ( 30 μm) at all times after injections.

The time span of the observable accumulated HRP in the neurons is much longer than that of $^{125}$I-NGF. Transport of HRP was observed in the dorsal horn and motor neurons. There was no labelling in this area of the spinal cord after injection of $^{125}$I-NGF. Hence, the rate of degradation of the transported NGF seems to be faster than HRP and that could account for the lack of transganglionic movement of NGF.

In summary, our study has shown that different populations of DRG neurons, in terms of sizes, were retrogradely labelled by NGF at different survival times. The total number of neurons which become labelled ( 85%) appears to be the same with NGF or HRP indicating that all sensory neurons retrogradely transport NGF. These results indicating that, in contrast to HRP, observing retrogradely labelled cells at any given time after $^{125}$I-NGF injection is not representative of the whole population of neurons capable of such transport. Whether this will prove a consistent difference between "receptor-mediated" transport versus "non-specific" transport is not known. (Supported by NS 18071).

272.5 WHEAT GERM AGGLUTININ BLOCKS THE BIOLOGICAL EFFECTS OF NERVE GROWTH FACTOR ON PC12 CELLS. G.E. Landreth and C. McCutchen. Dept. of Neurology, Medical University of South Carolina, Charleston, SC 29425.

The binding of nerve growth factor (NGF) to cell surface receptors initiates a variety of effects leading to the morphological and biochemical differentiation of a clonal pheochromocytoma line, PC12. The PC12 cells possess two interconvertible populations of NGF receptors, a low affinity, rapidly dissociating class (fast receptors) and a high affinity, slowly dissociating class (slow receptors). The occupancy of slow receptors is correlated with many of the biological effects of NGF. The NGF receptor is a membrane glycoprotein possessing N-acetylglucosamine residues which will bind the lectin, wheat germ agglutinin (WGA). It has previously been demonstrated that the binding of WGA to the NGF receptor results in the quantitative conversion of fast receptors to slow receptors, the acquisition of trypsin resistance by the hormone-receptor complex, and association of the receptor with the detergent-insoluble cytoskeleton. These characteristics are similar to those exhibited by slow receptors.

Treatment of PC12 cells with WGA dramatically and reversibly inhibited the ability of NGF to elicit three distinct biological effects. NGF causes the rapid production of ruffles on the cell surface; however, pretreatment of the cells with WGA blocked any NGF-induced change in cell surface morphology. NGF treatment stimulates the phosphorylation in situ of a 250 kDa cytoskeletal protein. Pretreatment of the cells with 50 µg/ml WGA inhibited the phosphorylation of this protein. The effect was reversible on addition of 10mM N-acetylglucosamine and was not produced by other lectins. PC12 cells also respond to NGF by extending neurites. If the cells are divested of their neurites, they will regenerate these processes over 24 hrs. If the cells are replated in serum-free medium, the lectin inhibits the ability of NGF to elicit neurite outgrowth. The inhibition is half-maximal at 3µg/ml WGA. The subsequent addition of 10mM N-acetylglucosamine reverses the effect of WGA, restoring the ability of NGF to elicit neurite outgrowth. Other lectins were without effect. These data demonstrate that the WGA-induced changes in the NGF-receptor interaction reflect important changes in the ability of the receptor to transmit biological signals, abrogating the biological effects of NGF on these cells.

272.6 NERVE GROWTH FACTOR AND TPA ALTER Nsp100 PHOSPHORYLATION IN PC12 CELLS IN A SIMILAR MANNER. T. Hama* and G. Guroff (SPON: L. Neckers). Section on Growth Factors, National Institute of Child Health and Human Development, NIH, Bethesda, MD 20205.

Treatment of PC12 cells with nerve growth factor alters several phosphorylations in the cells. One which has been looked at in some detail is the phosphorylation of soluble protein called Nsp100. Treatment with nerve growth factor causes a decrease in the phosphorylation of this protein in whole cells and also in cell-free extracts prepared from treated cells (End, Tolson, Hashimoto, and Guroff, J. Biol. Chem., 285: 6549, 1983). Nsp100 and the kinase acting on it have now been separated and partially purified (Togari and Guroff, J. Biol. Chem., 260: 3804, 1985).

In an effort to trace the biochemical events underlying this alteration in phosphorylation we have inspected the action of other effectors of phosphorylation on this system. Our data indicate that treatment of PC12 cells with TPA, a tumor promoter known to act on protein kinase C, also decreases Nsp100 phosphorylation. In addition, peptide mapping indicates that the pattern of decrease in the overall phosphorylation of this protein is the same as that seen with nerve growth factor treatment. And, as with nerve growth factor, the decrease is threonine-specific. The similarity between the effects of nerve growth factor and those of TPA in this system allow speculation on the role of protein kinase C activation as an early step in the action of nerve growth factor on phosphorylation in its target cells.

272.7 DECREASED FIBROBLAST GROWTH FACTOR SENSITIVITY OF NERVE GROWTH FACTOR-TREATED PC12 CELLS. K. Fujita*, H. Kuzuya*, and G. Guroff. (SPON: J. McLaughlin). Section on Growth Factors, National Institute of Child Health and Human Development, NIH, Bethesda, MD 20205.

Nerve growth factor treatment of PC12 cells leads to morphological and biochemical differentiation and to a decrease in cell division. A concommitant of the differentiation is a (heterologous) down-regulation of receptors for epidermal growth factor. Epidermal growth factor is a mitogen for most of the cells upon which it acts and for PC12 cells as well. We have suggested that the down-regulation is part of the mechanism by which nerve growth factor causes differentiation; that is, nerve growth factor prevents the action of mitogens to which the cells normally respond.

Fibroblast growth factor also acts on PC12 cells. It produces a form of morphological differentiation, but this differentiation is different than that produced by nerve growth factor (Togari, Dickens, Kuzuya, and Guroff, J. Neurosci., 5: 307, 1985). Fibroblast growth factor is a mitogen for many of the cells on which it acts. Our data show that fibroblast growth factor is a mild stimulant of PC12 cell division as well; both DNA content and $^{3}$H-thymidine incorporation are moderately, but definitely increased by the factor. Using orinthine decarboxylase activity as a probe, we have shown that treatment of PC12 cells with nerve growth factor decreases the action fibroblast growth factor on those cells. These data suggest that nerve growth factor down-regulates the fibroblast growth factor receptor. They support the general postulate that nerve growth factor causes a down-regulation of mitogen receptors.

272.8 NERVE GROWTH FACTOR AND OTHER AGENTS MEDIATE PHOSPHORYLATION AND ACTIVATION OF TYROSINE HYDROXYLASE THROUGH MULTIPLE PHOSPHORYLATION PATHWAYS. J. Cremins*, M. McTigue* and S. Halegoua* (SPON:M.Schwartz) Dept. of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, N.Y. 11794.

Nerve Growth Factor (NGF) and other agents rapidly stimulate different cellular kinases resulting in the phosphorylation of a number of proteins in PC12 cells (Halegoua and Patrick, Cell, 1980). In order to identify the kinases stimulated by NGF and to establish the second messenger systems involved in the generation of NGF responses, we further studied the phosphorylation and resultant change in activity of tyrosine hydroxylase (TH), a common substrate of multiple cellular kinases.

A rapid phosphorylation and activation of TH occured in PC12 cells in response to NGF, Epidermal Growth Factor (EGF), Bt$_2$cAMP, cholera toxin, $K^+$ depolarization, or phorbol ester (PMA). Examination of TH tryptic phosphopeptides analysed by two dimensional peptide mapping revealed four available sites of phosphorylation (T1-T4). Site specific phosphorylation of each of the four tryptic phosphopeptides was achieved in response to the various agents. NGF and cAMP elevation generated phosphopeptide patterns which were qualitatively the same (T1 and T3). Quantitatively, NGF resulted in a much greater phosphorylation of T3 than T1, while the reverse was true of cAMP elevation. T2 and T3 were phosphorylated in response to $K^+$ depolarization only in the presence of $Ca^{+2}$. EGF increased the phosphorylation of T3 and T4. PMA increased the phosphorylation of only peptide T3. The above data suggest the identity of NGF activated kinases as the Ca/phospholipid dependent protein kinase (C-kinase for T3), and cAMP dependent protein kinase (for T1).

In order to confirm that C-kinase is responsible for T3 phosphorylation, the following experiments were performed. Dose response using PMA indicated that it acted in the nanomolar range, comparable to its ability to activate C-kinase. Phorbol ester derivates which are unable to activate C-kinase also did not result in TH phosphorylation. A physiologically relevant activator of C-kinase, diacylglycerol, had the same effect as PMA. Inhibitors of C-kinase and cAMP dependent kinase were also used to elucidate the kinase identities involved in NGF action. Chlorpromazine and trifluoperizine, inhibitors of C-kinase, each blocked the phosphorylation of T3 but not of T1 in response to phorbol esters or NGF. These results demonstrate that the phosphorylation of T3 in response to NGF is due to C-kinase. The results using inhibitors indicate that the cAMP dependent kinase is regulated independently from C-kinase by NGF.

The above results demonstrate a molecular basis for the activation of TH by NGF and provide a framework for similar studies on other NGF actions.

272.9 EFFECT OF NERVE GROWTH FACTOR (NGF) AND SYMPATHECTOMY ON CELL SURVIVAL AND FIBER OUTGROWTH FROM SUBSTANCE P NEURONS GRAFTED IN OCULO. C. Ayer-LeLievre,* J. Kessler,[1] T. Ebendal*[2] and Å. Seiger* (SPON: W.A. Gregory). Dept. of Histology, Karolinska Institute, Stockholm, Sweden, [1]Dept. of Neurology, Albert Einstein College of Medicine, New York, and [2]Dept. of Zoology, Uppsala University, Uppsala, Sweden.

Nerve growth factor (NGF) is known to increase survival and SPLI content in late prenatal and postnatal dorsal root ganglion cells. Sympathectomy results in an increase of NGF levels in the rat iris.

Chronic injection of capsaicin (for 5 weeks) to young rats results in a disappearance of intrinsic substance P fibers. Cranial sensory (trigeminal, nodose petrous and superior-jugular) and parasympathetic (submandibular) ganglia containing substance P neurons have been transplanted from 11- to 21-day fetal to the eye chamber of capsaicin-treated rats. The outgrowth on iris of fibers from such grafted substance P neurons has been evaluated both qualitatively and quantitatively. Fetal central substance P neurons have also been grafted under the same conditions.

Grafts were treated with NGF, anti-NGF antibodies, or a saline solution as control. After a 3-week period in oculo, the NGF treatment resulted in a marked stimulation of the outgrowth of sensory substance P fibers which was not observed in untreated controls. Also, the strong NGF stimulation was selective for the sensory substance P-positive fibers as compared to sensory myelinated fibers and parasympathetic substance P-positive ones.

Sympathectomy of the host iris also resulted in an increase of substance P in fibers from grafted sensory ganglia, but the effects of sympathectomy and of NGF treatment were both quantitatively and qualitatively different.

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272.10 EFFECTS OF NERVE GROWTH FACTOR ON GRAFTS OF CHROMAFFIN TISSUE TO THE ANTERIOR CHAMBER OF THE EYE AND TO THE BRAIN. L. Olson,* I. Strömberg,* H. Björklund,*[1] T. Ebendal,*[2] M. Herrera-Marschitz*[3] and Å. Seiger* (SPON: L. Olson, Member ENA). Depts. of Histology and [3]Pharmacology, Karolinska Institute, Stockholm, [1]Dept. of Pharmacology, Pharmacia AB, Uppsala, and [2]Dept. of Zoology, Uppsala University, Uppsala, Sweden.

The possible effect of nerve growth factor (NGF) on survival and adrenergic nerve fiber production by heterologous grafts of adult adrenal chromaffin tissue to the anterior chamber of the eye and to the dopamine-denervated striatum was studied histochemically. A considerably larger area of the host iris became innervated by nerve fibers from the chromaffin grafts if the host iris was sympathetically denervated prior to grafting. This stimulation was probably caused by an increase in NGF levels in the iris and could be further enhanced by injection of NGF into the eye chamber. This treatment also greatly enhanced the density of the newly formed adrenergic nerves. Studies now in progress using dissociated cell suspensions from adult chromaffin tissue address the question as to which cell types respond to NGF. When chromaffin grafts are implanted in the dopamine-denervated striatum they are partly able to counteract the apomorphine-induced rotational behavior in such animals. Addition of NGF increases the number of permanently surviving catecholamine-containing cells in intrastriatal chromaffin grafts. A recently devised continuous infusion technique using a subcutaneously implanted osmotic minipump connected to a dialysis fiber stereotaxically placed in striatum permits continuous infusion of NGF over a period of one month. The chromaffin grafts then form rich networks of nerve terminals in host striatum directed towards the NGF source. The reductions of apomorphine-induced rotational behavior are much larger than without NGF treatment. Chronic infusion of NGF alone or of saline does not influence the apomorphine-induced rotations. The effects of chromaffin grafts plus NGF lasts for at least one year. Antibodies against glial fibrillary acidic protein (GFA) were used to monitor glial disturbances caused by the grafting and infusion techniques. Only minor or moderate changes of nearby astrocytes were seen. 6-Hydroxydopamine used to denervate host striatum caused a permanent, but transient, panstriatal gliosis.

(Supported by the Swedish MRC and NSRC.)

272.11 REGULATION OF NERVE GROWTH FACTOR (NGF) SPECIFIC MESSENGER RNA IN THE PERIPHERAL AND THE CENTRAL NERVOUS SYSTEM: R. Heumann*, S. Korsching* and H. Thoenen (SPON: H. Holländer). Dept. of Neurochemistry, Max-Planck-Institute for Psychiatry, D-8033 Martinsried,FRC.

We have developed a sensitive assay for the quantification of mRNA-NGF using cRNA-probes. An absolute quantification of mRNA-NGF was possible by adding unlabelled RNA synthesized in vitro as an internal standard to detect PNA recovery. Both NGF and its mRNA were correlated with the density of sympathetic innervation in the target organs of the sympathetic nervous system. However, while the superior cervical ganglia contained the highest levels of NGF, their mRNA was barely detectable. Thus, the high levels of NGF in sympathetic ganglia result from retrograde axonal transport rather than local synthesis.

Earlier studies in the central nervous system have shown that cholinergic magnocellular neurons are responsive to NCF. We have now found relatively high NGF and mRNA-NGF levels in the target regions innervated by these neurons (hippocampus: 1.4 ng NGF/g wet weight and 500 fg mRNA-NCF/µg poly(A)$^+$). In contrast, no mRNA-NGF was detectable in the region containing the cell bodies of these cholinergic neurons although the levels of NGF present in the same region was relatively high. These results support the concept that NGF acts also as trophic factor in the central nervous system.

Previous studies have indicated that a blockade of sympathetic axonal transport creates an increase in NCF levels in target organs. Iris cultures were therefore used to determine if this increase resulted exclusively from the inefficient removal of NGF after denervation or if innervating axons may also regulate NCF synthesis. The drastic increase of NGF-levels in cultured iris was preceeded by a mRNA-NGF increase starting at 2-3 h after explantation. Between 16 h - 24 h mRNA-NGF decreased again to a still elevated level at 48 h. The regulation in cultured iris was further investigated using inhibitors of transcription, translation or processing.

272.12 DENERVATION OF THE RAT IRIS DOES NOT INCREASE THE LEVEL OF mRNA ENCODING Beta NERVE GROWTH FACTOR. D.L. Shelton and L.F. Reichardt. Dept. Physiol. UCSF, San Francisco, CA. 94143

Beta nerve growth factor (NGF) is a protein necessary for the survival and maintenance of sympathetic and sensory neurons in vivo and in vitro. Evidence has accumulated which indicates that NGF is required at the growing tips of axons, and when present there, is bound, internalized, and retrogradely transported to the cell body. Recently it has been demonstrated that NGF is present in sympathetic target organs in vivo and that the amount of mRNA encoding for NGF (NGF mRNA) in a tisssue strongly correlates with the density of sympathetic innervation. This supports the hypothesis that NGF is produced by the target tissues of the responsive neurons.

Using the rat iris as a model target, it has been shown that the NGF content of a tissue increases dramatically after denervation or explant into culture. It is not known whether this change reflects a change in the synthetic rate of NGF, or is indicative of some change in the turnover of the molecule. In order to determine whether there is a change in synthesis controlled at the mRNA level, we have assayed the rat iris for its content of NGF mRNA after surgical and chemical denervation and after explant into culture.

Removal of the sympathetic innervation by either chemical or surgical methods has no effect on the level of NGF mRNA in the rat iris. The combined surgical removal of the sympathetic and sensory innervation also has no effect on the level of NGF mRNA in the iris. Therefore, denervation of the rat iris does not cause an increase in the content of NGF by increasing the level of NGF mRNA.

We also examined the NGF mRNA content of the rat iris after explant into culture. In this case, unlike the denervation in vivo, we did find a large, rapid increase in the NGF mRNA content of the rat iris. The increase was readily detectable within one hour, reached a maximum increase of ten to twenty fold by six to twelve hours, and was still evident after three days in culture. This increase did not reflect a general increase in the level of mRNA during explant. Although this response to explantation may be of use in understanding the normal control of NGF synthesis in vivo, it is not due to denervation. This differential effect of denervation and explantation indicates that organ culture may not be an acceptable model for denervation.

272.13 ABSENCE OF THE α AND γ SUBUNITS OF 7S NERVE GROWTH FACTOR IN DENERVATED RODENT IRIS: IMMUNOCYTOCHEMICAL STUDIES. R.A. Murphy, S. Landis+, J. Bernanke* and K. Siminoski*. Department of Anatomy and Cellular Biology and Department of Neurobiology+, Harvard Medical School, Boston, Massachusetts, 02115.

Immunocytochemical methods have been used to determine if denervated rodent iris produces nerve growth factor (NGF) in a form chemically similar to that of the 7S NGF complex in mouse submandibular glands. Irides of adult rats and mice were cultured for up to six days or sympathetically denervated by superior cervical ganglionectomy and left in situ four days. Tissues were stretched on slides and treated with rabbit antisera raised against the α, β and γ subunits of 7S NGF. Antibodies were visualized by indirect immunofluorescence. In control studies done on plastic (Poly/Bed 812) sections of mouse submandibular glands, antibodies of each of the subunits were localized to secretory granules of granular tubule cells. In rat iris, denervated 2-6 days, βNGF immunoreactivity was evident in a cellular plexus that closely resembles in distribution and morphology, nerve fibers in the normal iris, in agreement with a previous study (Rush, R.,Nature,312:364,1984). Identical staining patterns were observed in mouse iris. In neither rat nor mouse, however, did nerve-like processes stain with antibodies to the α and γ subunits nor was any other structure in the iris detectably immunoreactive for these proteins. This evidence suggests that the βNGF-like protein in denervated rodent iris is not synthesized as part of the 7S NGF complex, in contrast to mouse salivary gland. Cells in the iris also did not react with antibodies to epidermal growth factor, a protein that has been co-localized with NGF in mouse submandibular glands and in the prostate of the quinea pig.

272.14 EFFECTS OF CULTURE CONDITIONS ON THE CONCENTRATIONS OF NERVE GROWTH FACTOR (NGF) IN FIBROBLAST CELL CONDITIONED MEDIUM. M. Mowry* and N. J. Pantazis (SPON: W.W. Kaelber). Dept. of Anatomy, Univ. of Iowa Medical College, Iowa City, IA 52242

Although NGF is essential for development and maintenance of sensory and sympathetic neurons, little is known about factors which can affect NGF concentrations. Testosterone and thyroxine can increase NGF concentrations in the mouse submandibular gland. Fibroblast cells in culture synthesize NGF and secrete it into their feeding medium (conditioned medium). Isoproterenol is reported to increase NGF concentrations in fibroblasts in vitro (Schwartz, J.P. and Breakefield, X.O., Proc. Natl. Acad. Sci. USA, 77:1154, 1980). The present study examines the effects of various culture conditions on NGF concentrations in fibroblast cell conditioned medium (CM).

To test the effects of different media, confluent mouse fibroblast cells ($L_{929}$) were fed one of the following media: 1. Dulbecco's modified Eagle's medium/Ham's F12 (DMEM/F12); 2. N2 defined medium; 3. MCDB 105 defined medium. After 4d, the CM was removed, named cycle 1, and cells refed. This was repeated twice more at 6d intervals, and the CM named cycles 2 and 3, respectively. The CM was concentrated and an NGF radioimmunoassay was used to determine NGF concentrations. With DMEM/F12, the highest NGF concentrations were in the CM from cycle 1, whereas NGF concentrations in cycles 2 and 3 declined substantially. With MCDB 105, cycle 1 CM had an NGF concentration similar to cycle 1 CM obtained with DMEM/F12. In contrast to DMEM/F12, the NGF levels in cycles 2 and 3 did not drop with MCDB 105. Cells fed N2 medium had considerably lower levels of NGF in most cycles when compared to either DMEM/F12 or MCDB 105. Like DMEM/F12, the CM obtained with N2 showed a decline in NGF from cycle 1 through 3, with cycle 3 having no detectable NGF.

The effects of testosterone (10 and 1 nM), isoproterenol (100 and 10 μM) and thyroxin (10 and 1 μM) on NGF concentrations in fibroblast cell CM over three cycles were examined in DMEM/F12. Within each treatment group, cycle 1 had the greatest NGF concentration; cycles 2 and 3 had much less. When treatment groups were compared with controls (DMEM/F12 only), testosterone lowered the levels of NGF in CM from cycle 1; cycles 2 and 3 were similar between testosterone treatment and controls. In contrast, both isoproterenol and thyroxin increased NGF levels in cycles 2 and 3 respectively compared to controls. Neither agent had an effect on cycle 1.

In conclusion, MCDB 105 was best at maintaining higher levels of NGF in the CM. Testosterone, isoproterenol and thyroxin produced contrasting results; testosterone decreased NGF, whereas isoproterenol and thyroxin increased it. How these agents alter NGF levels, either by affecting NGF synthesis, degradation, or secretion, is unknown. (Supported by NIH grant GM28644 to NJP)

272.15 CARBOXYLMETHYLTRANSFERASE INHIBITORS BLOCK NERVE GROWTH FACTOR-MEDIATED INCREASE IN LEVELS OF TYROSINE HYDROXYLASE IN PRIMARY CULTURES OF BOVINE ADRENAL MEDULLARY CELLS. A. Acheson and H. Thoenen. Dept. of Neurochemistry, Max-Planck Institute for Psychiatry, 8033 Martinsried, W. Germany.

We have previously shown that primary cultures of calf chromaffin cells respond to nerve growth factor (NGF) treatment with selective increases in the levels of key catecholamine biosynthetic enzymes, including those of tyrosine hydroxylase (TH). The increase in TH levels occurs between hrs 36 and 48 of NGF exposure, and requires the continuous presence of NGF during the initial 36 hr.

It has recently been reported that several short- and long-term effects of NGF on PC12 cells, ranging from changes in membrane ruffling (minutes) to "priming" (days), can be inhibited by carboxylmethyltransferase inhibitors (Seeley et. al., JCB 98:417-426, 1984; Landreth and Rieser, JCB 100:677-683, 1985). We have examined the ability of several such inhibitors to block NGF-mediated TH increases. First, we measured the ability of these inhibitors to block the incorporation of $^{3}$H-methyl- versus $^{35}$S-methionine (met) into proteins. Cells were treated with 5'-deoxy-5'-methylthioadenosine (MTA, 3 mM), 5'-S-(2-methyl propyl) adenosine (SIBA, 1 mM), D-eritadenine (EA, 10 uM) or vehicle (DMSO) for 24 hr. Radioactive compounds were then given to the cells for 1 hr in the continued presence of the drugs. MTA and EA blocked the incorporation of $^{3}$H-methyl-met into protein by 65 and 80% respectively, while having no effect on $^{35}$S-met incorporation. SIBA blocked the incorporation of both isotopes into protein by 30%. When cells were treated with drugs for 24 hr, then left in culture in drug-free medium for an additional 24 hr, the effects of MTA and SIBA were fully reversed, whereas that of EA was only slightly reversed. Direct measurement of the formation of $^{3}$H-methyl esters via measurement of $^{3}$H-methanol formed upon alkaline hydrolysis of the protein also revealed extensive blockade of methylation after 24 hr by MTA and EA, but not by SIBA.

Cells were then treated concurrently with the inhibitors and NGF (1000 ng/ml) for 24 hr, then with NGF alone for an additional 24 hr. Under these conditions, MTA and EA blocked the NGF-mediated increase in TH levels, whereas SIBA was without effect. In addition, MTA and EA were unable to inhibit the cAMP-mediated increase in TH activity using the same drug treatment protocols, suggesting that the nature of their effects is selective. Given the apparent specificity of MTA and EA to block methylation, as well as the reversibility of MTA's effects, we conclude that methylation is important in NGF's mechanism of action for increased TH levels, and that the methylation event of importance occurs early in the process (first 24 hr).

272.16 DEVELOPMENT OF A HIGHLY SENSITIVE IMMUNOASSAY FOR MEASUREMENT OF ENDOGENOUS NGF IN PERIPHERAL AND CENTRAL NERVOUS SYSTEM OF THE ADULT RAT. G.Weskamp*, H.P.Lorez* and U.Otten (SPON: W.J.Fischli). Dept. of Pharmacology, Biocenter of the University, Basel, Hoffmann-LaRoche & Co.Ltd, Basel, Switzerland.

Nerve growth factor(NGF) is a trophic substance known to regulate the development and maintenance of function of sympathetic and sensory neurons. A sensitive enzyme-linked immunosorbent assay for NGF has been developed: Polyclonal goat antibodies against 2.5S mouseNGF were adsorbed to polystyrene multiwell plates. After adding the NGF-sample or standard solution, the second antibody, a specific monoclonal $IgG_{2a}$-antibody was applied. This antibody complex was detected with a biotin-labelled immunoglobulin to which peroxidase-coupled streptavidin was bound with high affinity. Enzyme-activity was quantified by the change in the optical density, using orthophenylenediamine as substrate. The detection limit of this assay is 0.1 pg/well corresponding to 0.004 fmol NGF.

Partial denervation of peripheral end-organs,e.g.iris, induced a significant, rapid increase in iris-NGF levels, which was followed by a prolonged hypertrophy of the remaining intact neurons, as monitored by biochemical and morphological analyses.

Determinations with this sensitive immunoassay revealed high levels of NGF in different brain regions, including the septum, hippocampus and neocortex, suggesting that NGF plays a physiological role in the functioning of the central nervous system.

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272.17 ASCITES DERIVED MONOCLONAL ANTIBODIES AGAINST NERVE GROWTH FACTOR OF HUMAN ORIGIN. K. Werrbach-Perez*, C. Beck*, L.W. Thorpe* and J.R. Perez-Polo (SPON: H. Eisenberg). Dept. of Biol. Chem. And Genetics, Univ. Tx. Med. Br., Galveston, Tx. 77550.

The nerve growth factor protein, NGF, is a developmental and maintainance factor for sympathetic and sensory neurons. Although similar in many respects, there are also differences among the species of NGF that may have important consequences as to the regulation of NGF activity in vivo. NGF can be isolated as $\alpha_2\beta\gamma_2$ multimer from mouse submaxillary gland, a $\gamma\beta$ complex from snake venom or an $\alpha\beta$ complex from human term placenta. The amino acid composition, isoelectric point and molecular weight of human term placenta β-NGF is very similar to that of murine β-NGF. Also, human term placenta β-NGF can displace murine β-NGF from NGF receptors found on IAN-1 human neuroblastoma cells. The clinical significance of NGF has yet to be elucidated since potent inhibitors of NGF activity hamper the measurement of NGF activity in human sera and radioimmunoassays for NGF protein in human sera have relied on antibodies directed against mouse β-NGF. NGF of human origin, whether from term placenta or secreted by a human neurofibroma line, is antigenically indistinguishable. The goal of this project was to develop stable hybridoma clonal lines compatible with growth in ascites fluid that would secrete high affinity antibodies to human β NGF. Using a modification of in vitro immunization techniques developed by us, 454 viable hybridoma cultures were obtained after fusion with P-3 $IgG_1$, secreting mouse myeloma. Of these, after ELISA screening against human β-NGF and cloning, there were six clones that proved to be stable. Ammonium persulfate precipitate of conditioned media of all six clones was found to be active against human beta-NGF. These results were confirmed by immunoblotting and immunoprecipitation with $^{125}$I-human β-NGF. Antibodies secreted by clones PP3, 5 and 6 are $IgG_1$ while 10, 11 and 12 secrete $IgG_1$ and IgM antibodies. P-3 is an $IgG_1$ secretor; thus, both the myeloma $IgG_1$ and IgM gene from spleen are expressed and three classes of antibodies are secreted: IgG and IgG/IgM hybrid. This interpretation is consistent with immuneprecipitation results with protein A and a second antibody, protein-A combination that binds both IgG and IgM. Of these PP3 grows well in ascites fluid and is useful at dilutions of $10^5$. (Supported by NIH grant NS 18708 and Robert Welch Foundation Grant H-698).

## TRANSMITTERS IN INVERTEBRATES III

273.1 DIFFERENTIAL DISTRIBUTION OF TWO HOMOLOGOUS NEUROPEPTIDES (RPCH & AKH) IN CRAYFISH NERVOUS SYSTEM. A.J. Madsen, Jr.*, W.S. Herman* and R. Elde (SPON: G. Giesler, Jr.). Departments of Anatomy, Genetics and Cell Biology, University of Minnesota, Minneapolis, MN 55455.

Red pigment concentrating hormone (RPCH; pQLNFSPGW-$NH_2$) and adipokinetic hormone (AKH; pQLNFTPNWGT-$NH_2$) are peptides isolated from neurohemal tissues of arthropods. Because of their striking structural similarities, these peptides have been regarded as members of a family of invertebrate peptides. The present study was undertaken to determine if immunoreactivity related to each of these peptides coexists within single neurons of a representative arthropod, the crayfish, or whether neurons differentially express peptides detected by antisera selective for RPCH and AKH.

Crayfish (Orconectes immunis) brains and eyestalks were fixed by immersion in Zamboni's fluid, sectioned at 10um on a microtome-cryostat and collected on gel-coated slides. Sections were rehydrated and incubated in primary antisera directed against RPCH or AKH, or absorption control serum for 12-16 hours at 4° C. After washing the sections, fluorescein labeled goat anti-rabbit IgG was applied for 60 minutes. Sections were rinsed, counterstained with ethidium bromide and coverslipped. Previous studies using the immunoblotting technique of Larsson (1981) indicated the antisera to be selective for their respective peptides and exhibit little, if any, cross-reactivity for the heterologous peptide.

Nerve fibers and terminals containing RPCH- or AKH-immunoreactivity were abundant in many regions of the eyestalk and brain of the crayfish. Neuronal cell bodies containing RPCH- or AKH-immunoreactivity were observed throughout the eyestalk, the protocerebrum, the deutocerebrum and the tritocerebrum. Examination of serial sections indicated that individual neurons stained for either AKH-immunoreactivity or RPCH-immunoreactivity, but not both. These results strongly suggest that although RPCH and AKH share strong sequence homologies, individual neurons are dedicated to the production of either one or the other of these two peptides. The mechanisms by which the differential production of AKH and RPCH might occur are not understood, but two possibilities seem likely. Firstly, mRNA may code for separate prohormones which do not coexist in single neurons of the crayfish. Alternatively, post-translational processing of a common biosynthetic precursor may give rise to the production of one peptide at the expense of the other.

Supported by the Graduate School of the University of Minnesota and the Minnesota Medical Foundation.

273.2 INDIVIDUAL NEUROSECRETORY CELLS REGULATING CORPUS ALLATUM ACTIVITY IN THE BRAIN OF THE LOCUST SCHISTOCERCA AMERICANA. M. Zaretsky Dept. of Elect. Engr. and Comp. Sci., Univ. of California, Berkeley CA 94720.

Juvenile hormone (JH) is the principal gonadotropin for most insects. Among several effects of JH upon oogenesis, the best understood is control of production of the protein vitellogenin by the fatbody. A number of reports on several insect species indicate the neurosecretory cells (NSCs) of the brain are significantly involved in the regulation of JH synthesis and oogenesis. The number and location of the NSCs involved varies with the insect group. Both excitatory and inhibitory interactions of the NSCs with the glands that synthesize JH, the corpora allata (CA), have been reported. There is strong evidence for allatotropic hormones, both in the general control of molting and metamorphosis and in specific control of oogenesis and yolk deposition, but these have been neither isolated nor characterized. It is not known what determines the changes in the rate of JH synthesis.

The locust Schistocerca is highly advantageous as an experimental animal for the study of JH regulation. The innervation of the CA is excitatory and comes mainly from the lateral neurosecretory cells (LNSCs) of the brain. The axons of these neurons leave the brain for the corpus cardiacum (CC) by way of NCCII and pass on to the CA in NCAI. To identify the neurons of the brain that excite JH synthesis by the CA, the NCAI was back-filled with cobalt. The axons within NCAI were examined with the electron microscope. Individual NSCs of this group of neurons were filled intracellularly with Lucifer Yellow dye or horseradish peroxidase, which reveal their individual morphology. To correlate the spontaneous activity of these NSCs with the gonotrophic cycle, extracellular recordings were made from the nerve to the CA, and the activity of individual cells was monitored with intracellular electrodes.

In addition to these techniques, stimulation of individual NSCs that innervate the CA will be combined with radiochemical assays for JH [Tobe SS and Pratt GE(1975) J. exp. Biol. 62:611]. Thereby, it should be possible to identify individual NSCs that regulate JH synthesis by the CA. Of particular interest is the effect of circulating levels of JH and ecdysteroids upon the NSCs that regulate JH synthesis.

273.3 IMMUNOHISTOCHEMICAL LOCALIZATION AND PHYSIOLOGICAL ACTIONS OF TWO RELATED INSECT NEUROPEPTIDES (CC1 AND CC2) M.E. Adams, M.F. Ray*, R.K. Ho*, S.J. Kramer*, and P. Marbach*, Research Laboratories, Zoecon Corp., Palo Alto CA 94304 and Pharmaceutical Div., Sandoz Ltd., Basel, Switzerland.

Two peptide hormones occurring in a prominent neurohemal organ, the corpus cardiacum of the cockroach (Periplaneta americana) have been isolated and sequenced. They are blocked octa-peptides referred to here as "CC1": pGluValAsnPheSerProAsnTrpNH$_2$ and "CC2": pGluLeuThrPheThrProAsnTrpNH$_2$, (Scarborough, et al., PNAS 81: 5575, 1984). The identical structures have been independently reported as "M1" (CC1) and "M2" (CC2) (Witten, et al., Biochem. Biophys. Res. Comm. 124: 350, 1984) or as "Neurohormone D" (CC1) (Baumann, et al., Biomed. Biochim. Acta 43: K13, 1984). CC1 and CC2, the locust adipokinetic hormones (AKH I, II) and crustacean red pigment concentrating hormone (RPCH) comprise a family of peptides with close sequence homology. All stimulate muscle rhythmicity and modulate carbohydrate and lipid metabolism. We report here that CC1- and CC2-like immunoreactivity (CC-IR) is not confined to the corpus cardiacum, but also occurs in neurons throughout the nervous system and in nerve terminals innervating the gut, where they possibly function as neurotransmitters.

Serum antibodies were produced in rabbits against amino-terminal CC1 or CC2 conjugates to BSA. Radioimmunoassay studies conducted thus far indicate that the antisera cross-react between CC1 and CC2, but do not recognize AKH or RPCH. Immunohistochemical staining shows strong CC-IR in the corpora cardiaca, consistent with release of the peptides as circulatory neurohormones. CC-IR also occurs in neurons of the brain and segmental ganglia. Brain neurons occur in the medial and lateral pars intercerebralis and in the optic lobes. In the segmental ganglia, immunoreactive medial and lateral cell pairs as well as large medial unpaired neurons are evident. Central arborizations in the segmental ganglia occur in the ventro-medial neuropile areas typically innervated by neurosecretory cell types. The presence of CC-IR nerve terminals innervating foregut musculature suggests synaptic transmitter roles for the peptides. Application of $10^{-7}$ M CC1 or CC2 to the gut region innervated by these terminals causes rhythmic contractions and initiation of peristaltic movements.

Our studies implicate CC1 and CC2 in close-range transmitter functions as well as in hormonal roles. The cellular mechanisms underlying CC1/CC2 actions are under investigation.

273.4 IMMUNOCYTOCHEMICAL MAPPING OF PEPTIDES IN THE BRAIN AND SUBESOPHAGEAL GANGLION OF *MANDUCA SEXTA*. U. Homberg*, S.G.Hoskins, and J.G. Hildebrand (SPON: Tim Kingan). Dept. Biol. Sci., Columbia Univ., New York, NY 10027.

Neuropeptides can be detected in the insect brain not only in the neurosecretory cells of the *pars intercerebralis* but also in other regions [Duve & Thorpe, in *Insect Neurochemistry and Neurophysiology* (eds. A.B.Borkovec & T.J. Kelly), Plenum (1984); Schooneveld et al., *Gen. Comp. Endocrinol. 42*:526 (1985)]. As part of our continuing study of the distribution of putative neurotransmitters in the brain, and especially the antennal lobes, of *Manduca* [Hoskins & Hildebrand, *Soc. Neurosci. Abstr. 9*:216 (1983); Hoskins et al., *Soc. Neurosci Abstr. 10*:688 (1984)], we have investigated the distribution of immunoreactivities recognized by antisera specific for locust adipokinetic hormone (AKH), cholecystokinin (CCK), FMRFamide, glucagon, insulin, leu- and met-enkephalins, substance P, and somatostatin.

Particular sets of neurons in the *pars intercerebralis* are stained reproducibly by each of the antisera except those specific for met-enkephalin and somatostatin; those antisera reacted with none of the cells. Some of the immunoreactive neurons have arborizations in the protocerebrum and tritocerebrum and, in the case of the AKH-immunoreactive cells, axons that can be traced into the neurosecretory *corpora cardiaca*. In other parts of the brain and subesophageal ganglion, immunoreactivities related to AKH, FMRFamide, CCK, and substance P are detectable. In the optic lobes about 50 cell bodies associated with the *lobula* and *medulla* show AKH- or substance-P-like immunoreactivity. The antennal lobes exhibit 10-15 local interneurons immunoreactive to anti-AKH, a few local interneurons immunoreactive to anti-substance P, and one cell immunoreactive to anti-CCK. In the median protocerebrum several neurons showing AKH- or substance-P-like immunoreactivity can be detected in the upper division of the central body and the diffuse protocerebral lobes. The subesophageal ganglion has 2 pairs of neurons with substance-P-like immunoreactivity and 5 that stain with anti-AKH, one of which projects into the antennal lobes and further into the protocerebrum. Experiments in which alternate sections were exposed to different antisera revealed that glucagon- and CCK-like immunoreactivities are present in some of the same cells in the *pars intercerebralis*. Some but not all of the neurons stained by anti-AKH are also immunoreactive to substance P antisera and vice versa. These observations suggest that either these antisera recognize different epitopes on a single peptide or the immunoreactive cells contain more than one neuropeptide.

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273.5 IDENTIFICATION OF APLYSIA NEURONS WITH CCK/GASTRIN-LIKE IMMUNOREACTIVITY IN WHOLEMOUNTS OF APLYSIA GANGLIA. J. K. Ono, Division of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA, 91010.

An immunohistochemical survey of wholemounts of the CNS of the marine mollusc, Aplysia californica with antisera against the peptides cholecystokinin (CCK) and gastrin demonstrates that numerous Aplysia neurons contain CCK/gastrin-like peptides. Immunoreactive neurons were present in all ganglia except for the pleural ganglia and immunoreactive fibers were observed in the intestine. Several of these immunoreactive neurons in the buccal and cerebral ganglia are re-identifiable. By correlating physiological characteristics with immunoreactivity, buccal neurons B13 and B7, originally identified by Gardner (J. Neurophysiol., 40:349, 1977), have been shown to contain CCK/gastrin-like immunoreactivity. Radioenzymatic assays of individual neurons for acetylcholine (ACh) indicate that B13 contains significant concentrations of ACh, comparable to the levels in the previously identified cholinergic buccal neurons, B4 and B5. The cerebral A cluster neurons, originally described by Fredman and Jahan-Parwar (Brain Res., 100:209, 1975) also display CCK/gastrin-like immunoreactivity. However, application of mammalian forms of CCK and gastrin to the cerebral B cluster neurons, which are postsynaptic to the A neurons, does not elicit a conductance change comparable to the synaptic response.

Variations in the staining patterns with different antisera to CCK and gastrin were observed. Only antisera which recognize the carboxyl terminus common to both CCK and gastrin (WMDF-amide) appear to stain Aplysia neurons. Among the antisera which recognize the Aplysia form of CCK/gastrin, only one (antiserum 5135) stained neurons in the ventral lower right quadrant of the abdominal ganglion. Previously, Osborne et al. (Science, 216:409, 1982) demonstrated that the serotonergic giant cerebral neurons in another mollusc, Helix, contains a CCK-like peptide. However, the cell body of the homologous neurons in Aplysia, the metacerebral or C1 neurons, do not appear to be immunoreactive to the various antisera to CCK and gastrin.

These studies suggest that in the Aplysia CNS there may be more neurons displaying CCK/gastrin-like immunoreactivity than those that are positive for 5HT (Ono and McCaman, Neurosciences, 11:549, 1984). Moreover, the peptide may be co-localized with conventional neurotransmitter amines in certain neurons. The Aplysia form of the CCK/gastrin peptides may be similar to mammalian forms of CCK and gastrin at the caboxyl end but may differ toward the amino end. (Supported by NIH grant NS 18862)

273.6 FMRFAMIDE-LIKE SUBSTANCES IN THE SWIM MOTOR NEURONS OF THE LEECH. B. J. Norris* and R. L. Calabrese. The Biological Laboratories, Harvard Univ., Cambridge MA 02138.

The neuropeptide FMRFamide (Phe-Met-Arg-Phe-NH$_2$), first identified in molluscs (Price and Greenberg, Science 197:670-671, 1977), has been localized with immunocytochemical techniques to a distinct set of neurons in the central ganglia of the leech Hirudo medicinalis (Kuhlman et al., Neurosci. Abs. 46.2, 1984). The excitatory motor neurons that innervate the longitudinal muscles of the body wall appear to be among those that contain FMRFamide-like immunoreactivity (FLI).

We labeled identified longitudinal motor neurons with intracellular injections of Lucifer yellow and tested them for FLI using indirect immunofluorescent labeling. All identified excitatory longitudinal motor neurons on the dorsal surface of the ganglion showed FLI. Cells 5, 6, 8, 107, 108 and L labeled intensely while cells 3 and 4 labeled only faintly. A putative excitatory longitudinal motor neuron on the ventral surface of the ganglion (cell 157) did not show FLI. None of the inhibitory longitudinal motor neurons (cells 1, 2, 7, 9 and 102) showed FLI. Two motor neurons innervating the medial dorsoventral muscles also contained FLI. Cell 117, an excitatory neuron, labeled intensely and cell 101, an inhibitory neuron, labeled very faintly. We will determine the amount of FMRFamide-like substance in a single identified longitudinal motor neuron (cell L) using radioimmunoassay (Li and Calabrese, this meeting).

Our preliminary experiments show that bath applied FMRFamide at a concentration of 0.8 x 10-7 mM caused a strong contraction in longitudinal muscle. Continuing research involves characterizing the effects of applied FMRFamide on longitudinal muscle.

The major excitatory neuromuscular transmitter in the leech is acetylcholine (Wallace, J. Neurosci. 2:1108-1118, 1982), and excitatory neuromuscular transmission is blocked by curare (Kuffler, J. Comp. Physiol. 124:333-338, 1978). We will block cholinergic transmission with curare and look for residual neuromuscular effects which could be due to a FMRFamide-like peptide.

We wish to thank T. O'Donohue and W. Watson for their generous gift of anti-FMRFamide antiserum. This work is supported by NIH grant RO1 NS21232.

273.7 FMRFamide-LIKE SUBSTANCES IN THE LEECH: BIOCHEMICAL CHARACTERIZATION. C. Li and R. L. Calabrese. Biological Laboratories, Harvard University, Cambridge, MA 02138.

FMRFamide is a tetrapeptide that is present in a variety of invertebrates and vertebrates. FMRFamide-like immunoreactivity has been localized to a number of neurons and neural processes in the leech, Hirudo medicinalis (Kuhlman, Li, and Calabrese, 1985a, J. Neurosci., in press). To determine whether true FMRFamide exists in the leech, acidified methanol extracts of leech nerve cords and heart tubes were examined biochemically for FMRFamide-like substances. Using a radioimmunoassay, 8-15 pmol, 2-3.5 pmol, and 1-4 pmol of FRMFamide-like equivalents were measured per nerve cord, head brain, and heart tube extract, respectively. The inhibition of trace binding to anti-FMRFamide antibody (a kind gift from T. O'Donohue and W. Watson) by serial dilutions of synthetic FMRFamide and tissue extracts was parallel, indicating a similar affinity of synthetic FMRFamide and tissue extracts to the antibody.

Extracts of nerve cords and heart tubes were further purified on a $C_{18}$ Sep-pak cartridge for reverse phase high pressure liquid chromatography (HPLC) analysis. Five peaks containing FMRFamide-like immunoreactivity were recovered from nerve cord extracts. The majority of immunoreactivity was found in three peaks. The largest of these peaks co-eluted with synthetic FMRFamide; the other two peaks co-eluted with oxidation products of synthetic FMRFamide. Heart tube extracts contained two peaks that were FMRFamide-like immunoreactive. The major peak co-eluted with synthetic FMRFamide, and the minor peak co-eluted with one of FMRFamide's oxidation products. We are isolating the peptides for amino acid sequencing.

A pair of neurons innervating the heart, the heart modulatory neurons (HA cells), were found to contain FMRFamide-like immunoreactivity using immunocytochemical methods; in addition, the peripheral effects of bath application of FMRFamide are very similar to those effects produced by HA cells (Kuhlman, Li, and Calabrese, 1985b, J. Neurosci., in press). HA cells were isolated to quantify and analyze the FMRFamide-like immunoreactivity. 20-50 fmol of FMRFamide-like equivalents were measured per HA cell extract. HPLC analysis of cell extracts revealed two peaks of immunoreactivity, one that co-eluted with synthetic FMRFamide and one that co-eluted with an oxidation product of FMRFamide. These data support our hypothesis that HA cells use FMRFamide as their primary neurotransmitter.

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273.8 IMMUNOCYTOCHEMICAL LOCALIZATION OF SEROTONIN, FMRFamide, BPP, AND $SCP_B$ IN NUDIBRANCH MOLLUSKS. Roger D. Longley* and Alison J. Longley. Pacific Sciences Institute, P.O. Box 835, Friday Harbor, WA 98250 and Friday Harbor Laboratories, Friday Harbor, WA 98250.

We have used antibodies to serotonin, FMRFamide, BPP, and $SCP_B$ in a wholemount method which allows efficient, direct visualization of the three dimensional structure of immunoreactive somata, axons, and neuropil in the nudibranch central nervous system. $SCP_B$ containing neurons were identified with a mouse monoclonal antibody marked with a rhodamine labeled second antibody, which allowed crossreactivity comparisons in the same preparation to other antibodies labeled with FITC. We tested this antibody for specificity by first filling, with lucifer yellow, visually identifiable $SCP_B$ containing neurons in the Tritonia buccal ganglia before processing with the $SCP_B$ antibody.

In the species examined [Hermissenda (Phidiana) crassicornis, Aeolidia papillosa, Armina californica, and Tritonia diomedea], strong homologies were present in soma location and axon distribution for several large neurons. The serotonergic giant cerebral neurons (GCNs) and an unpaired serotonergic left pedal neuron, apparently LPd1 in Tritonia and Hermissenda, are uniformly present. The GCNs and LPd1 can be recognized with antiserotonin antibody in developing Hermissenda (AJ Longley, Neurosci. Abstr., 1985). In each species, a large $SCP_B$ immunoreactive neuron in the cerebral ganglion, usually adjacent to the GCN, sends axons into the cerebral nerves. This neuron is especially prominent in Aeolidia. In the buccal ganglion, a large $SCP_B$ neuron with an axon in the gastroesophageal connective was seen. The gastro-esophageal ganglion also contains a large $SCP_B$ neuron with an axon in the gastric nerve. With the antibodies used, many other neurons, both large and small, could be seen for which cross species homologies were not evident. In Hermissenda, a large neuron in the right pleural ganglion, apparently the largest neuron in this ganglion, is immunoreactive to $SCP_B$, but a comparable neuron was not seen in the other species examined.

The following general observations were made. 1) BPP immunoreactive neurons crossreact with antiFMRFamide antibody. 2) Axons immunoreactive to antiFMRFamide were not seen in peripheral nerves. 3) Some FMRFamide and $SCP_B$ immunoreactive neurons crossreact. 4) The largest number of immunoreactive neurons seen contained serotonin and $SCP_B$, but there are ganglionic differences in the distribution of serotonin and $SCP_B$ containing neurons. Buccal and gastroesophageal neurons contain $SCP_B$ and a small number of $SCP_B$ neurons are in the pleural ganglia, but there are no serotonergic somata in these ganglia. Most of the serotonergic neurons are in the pedal ganglion, but there are only about two $SCP_B$ neurons in that ganglion. 5) Serotonin and $SCP_B$ containing neurons in the cerebral ganglia innervate the rhinophore ganglia with densely varicose endings.

273.9 EFFECTS OF NEUROTENSIN AND A NEUROTENSIN-RELATED PEPTIDE FROM LOBSTER ON CARDIAC FUNCTION. S.R. Kirschenbaum, C.A. Aghajanian*, C.H. Price, and R.E. Carraway*. Department of Biology, Boston University, Boston, MA 02215 and Department of Physiology, University of Massachusetts Medical Center, Worcester, MA 01605.

Many peptides isolated from vertebrates have been demonstrated in invertebrates by radioimmunoassay and immunohistochemistry although few precise structures and functions have been identified. We previously reported the occurence of neurotensin (NT)-related peptides in the heart and hepatopancreas of the lobster (Homarus americanus). The partial sequence of one of these peptides shows close sequence homology with the C-terminal region of mammalian-NT. Using mammalian NT and partially purified lobster NT we examined the role of NT in lobster cardiac function .

Heart tone, beat frequency and amplitude were recorded from an intact heart isolated from CNS inputs and perfused with saline to which cardioactive substances were added. Perfusion with $10^{-5}$ M NT for several seconds induced a dose-dependent increase in muscle tone and a decrease in beat amplitude within seconds and a delayed increase in frequency . All effects were reversible: after washout of NT, the heart returned to baseline tone and amplitude in about 3 minutes and baseline frequency in 10-15 minutes. Repeated applications resulted in reduced maximal responses indicating desensitization to NT.

Intracellular recordings from single muscle fibers were made to further examine the effect of NT on muscle. NT ($10^{-5}$ M) depolarized the resting membrane potential by 10-20 mV and decreased the magnitude of the rhythmic depolarizations. These dose-dependent responses had the same onset and washout times as responses in the intact heart. A synthetic peptide having the proposed sequence for lobster immunoreactive NT induced similiar depolarizations of the muscle membrane at concentrations of about $10^{-9}$ M.

These results suggest that the NT-like peptides demonstrated in lobster heart play a role in invertebrate cardiac function. The lobster immunoreactive NT would thus be structurally as well as functionally related to mammalian NT for which a cardiovascular regulatory role has been proposed.

273.10 PARTIAL PURIFICATION OF A POLYPEPTIDE FROM LOBSTER EYESTALKS THAT INDUCES cGMP ACCUMULATION IN LOBSTER NEUROMUSCULAR PREPARATIONS. M.F. Goy*, C.M. York*, and D. Mandelbrot* (SPON: D. Potter) Neurobiol. Dept., Harvard Med. School, Boston, MA 02115.

We are investigating the function of cyclic GMP (cGMP) at the neuromuscular junction of the lobster, Homarus americanus. Several observations suggest that this nucleotide might play a role in regulating some aspect of crustacean neuromuscular physiology: 1) lobster muscle is among the richest known sources of cGMP-dependent protein kinase, 2) the tissue contains several phosphoproteins whose state of phosphorylation can be influenced more effectively by cGMP than by cAMP, and 3) guanylate cyclase and phosphodiesterase are highly active in these preparations.

Although previous work has shown that the physiological properties of these synapses can be modulated by a number of circulating neurohormones, no factor has yet been identified in the lobster that alters muscle cGMP levels. Accordingly, we screened extracts of neural and neurosecretory structures for the ability to promote cGMP accumulation in isolated exoskeletal muscles. Extracts of the eyestalk (which includes the neurosecretory sinus gland) contain a factor that causes greater than 100-fold increases in muscle cGMP content, whereas extracts of other tissues are inactive.

The biological activity of the eyestalk factor is destroyed by boiling and by digestion with trypsin, chymotrypsin, or pronase. The factor has been carried sequentially through several purification steps, including differential centrifugation, gel filtration chromatography, ion exchange chromatography, and reverse phase chromatography, with a net purification of greater than 13,000-fold over the starting material. These procedures reveal that the factor has an apparent molecular weight of 6-10,000 daltons, an acidic isoelectric point, and hydrophobic binding characteristics. In other crustacea, a peptide with a similar molecular weight and an acidic isoelectric point (the crustacean Hyperglycemic Hormone) has been implicated in the cGMP-mediated regulation of glycogen metabolism. However, in lobsters, the hyperglycemia-inducing factor is reported to have a molecular weight of 1,500 daltons. Our long range goal is to purify the lobster eyestalk factor described here to homogeneity, compare it to the hyperglycemic hormones of other crustacea, and use it as a probe to evaluate the physiological role of cGMP at the neuromuscular junction. (Supported by NIH grant #NS 07848 to E.A. Kravitz. Lobster eyestalks were kindly donated by Legal Seafood, Boston, MA).

273.11 OCTOPAMINE- and cAMP-STIMULATED PHOSPHORYLATION OF A PROTEIN ASSOCIATED WITH PHOTORECEPTOR SOMATA IN THE LIMULUS VENTRAL EYE. S. C. Edwards* and B-A. Battelle. C. V. Whitney Laboratory, Univ. of Florida, St. Augustine, FL 32086

Efferent innervation to retinas exists in many animal species. The Limulus ventral eye provides an excellent system for studying the role of efferent innervation in visual function. Previous studies (reviewed by Battelle, TINS 7:277, 1984) have shown that in the ventral eye, the efferent fibers project specifically to the photosensitive membrane of the photoreceptors. The biogenic amine octopamine (OCT) fulfills many criteria as a neurotransmitter within these fibers. O'Day and Lisman (ARVO, 1984) showed that exogenously applied OCT increases the rate of dark adaptation of the photoreceptors. These effects were mimicked by forskolin, a nonspecific adenylate cyclase stimulator, and cAMP.

We are studying the biochemical effects of OCT on Limulus ventral photoreceptor cells. Since OCT stimulates an increase in the level of intracellular cAMP and activates adenylate cyclase in these cells (Kaupp et al., Vision Res. 11:1503, 1982), we speculated that OCT may cause changes in the phosphorylation of photoreceptor proteins. We have examined the effects of OCT on the phosphorylation of proteins in ventral eye preparations enriched in intact photoreceptor cell bodies (P-fraction) and in axon-enriched fractions which contain only few photoreceptor cell bodies (A-fraction). The fractions are maintained at 4°C in Limulus saline containing glucose for 12-18 hrs following dissection, then preincubated in the light in saline containing [32P] orthophosphate (2.3 mC/ml) for 1.5 hrs prior to experimentation. We have reported (ARVO, 1985) that subsequent exposure of P-fractions to OCT (0.2 - 2.0 uM) stimulates the phosphorylation of a protein with an apparent molecular weight of 122 ± 2.3 kD based on its mobility on SDS-PAGE and detection using autoradiography. Enhanced phosphorylation of the 122 kD protein occurred within 5 min of exposure of the tissue to OCT and was blocked when the tissue was preincubated for 10 min with 10 uM phentolamine, an OCT receptor blocker, prior to exposure to OCT (2 uM) plus phentolamine. OCT did not stimulate the phosphorylation of this protein in the A fraction.

We now report that the 122 kD protein is phosphorylated when the P-fractions are exposed for 5 min to either 10 uM 8-bromo-cAMP or 10 uM forskolin. Furthermore, this protein and proteins having approximate molecular weights of 170 and 57 kD are phosphorylated when homogenates prepared from either the P- or A-fractions are exposed to 10 uM 8-bromo-cAMP in a MOPS buffered medium (pH 7.0) containing gamma-labelled [32P] ATP, 10 mM $MgCl_2$, 1 mM EGTA, and 1 mM DTT. These results suggest that the OCT-stimulated phosphorylation of the 122 kD protein in the ventral eye is mediated by cAMP and probably involves a cAMP-dependent protein kinase. (Supported by NIH Grant #EY05724-01 and the C. V. Whitney Laboratory).

273.12 AUTORADIOGRAPHIC LOCALIZATION OF SEROTONIN RECEPTORS IN APLYSIA GANGLIA. M.J. Kadan and P.R. Hartig, Dept. of Biology, The Johns Hopkins University, Baltimore, MD 21218.

Serotonin and dopamine receptors have been identified in Aplysia by $^3$H-LSD binding to ganglia homogenates (Drummond et al., Brain Res. 184: 163-177 (1980)). We have used the sensitive serotonergic ligand $^{125}$I-LSD to study the distribution and properties of receptor sites in the Aplysia nervous system. $^{125}$I-LSD is 120 fold more sensitive than $^3$H-LSD for receptor assays, which allowed us to carry out binding studies on a single ganglion and to generate autoradiographic images of receptor distribution with 1-5 day exposures.

Ten micron frozen sections were cut from Aplysia ganglia and thaw mounted on gelatin subbed glass slides. Sections were covered with ligand solution (0.3 nM) ± competing drugs for 20 min. at 37°C, cooled, then washed 40 min. in ice cold water. Sections were blown dry under a stream of cold air and exposed to film (LKB Ultrofilm or Kodak AR10 stripping film). Binding was assessed by microdensitometry of the autoradiographic image, or by wiping sections from the slides and directly counting the radioactivity.

$^{125}$I-LSD binding to ganglia sections was saturable and stereospecific. Cinanserin, an effective serotonergic antagonist in this system, reached a plateau of displacement near 20 µM after displacing 65-75% of the bound $^{125}$I-LSD. Serotonin was the most potent agonist displacer, reaching a plateau near 100 µM. Dopamine was a weaker displacer than serotonin while epinephrine and norepinephrine showed no significant displacement at 100 µM. Interestingly, 8-OH-DPAT, a selective serotonergic ($5\text{-}HT_{1a}$) agonist appeared equal to or more potent than serotonin as a displacer.

Comparison of autoradiographic images and histologically stained sections demonstrated that the specific binding was restricted to the neuropil region. No specific labeling of the surrounding cell soma or ganglion sheath was observed. This receptor distribution matches electrophysiological data on serotonergic responses in this system. Specific labeling of the axonal processes within the sheath of the ganglia interconnective was also observed.

This study provides the first autoradiographic mapping of serotonin receptor distribution in Aplysia, demonstrating that receptor binding sites within the ganglia are localized to the neuropil region, in accord with electrophysiological data.

273.13 HISTOFLUORESCENCE OF CATECHOLAMINE-CONTAINING NEURONS IN *Drosophila*. V. Budnik*, A.M.Vallés* and K. White* (SPON: H. Epstein). Department of Biophysics and Department of Biology, Brandeis University, Waltham MA 02254.

The neurotransmitter dopamine has been identified in several invertebrates. In the fruit fly *Drosophila melanogaster*, it is possible to alter the levels of dopamine using genetic variants in the gene *Ddc* encoding the enzyme dopa decarboxylase (Wright et al., Genetics, 84: 287, 1976). As a prelude to the study of effect of dopamine concentration on neural development, we have initiated a study of catecholaminergic neurons in *Drosophila*. In order to identify dopamine containing neurons, the pattern of catecholamine containing neurons in the fly central nervous system (CNS) was determined using two histofluorescence techniques (Flanagan, J. Insect Physiol., 30: 697, 1984; Furness et al, Histochem, 57: 285, 1974). The larval CNSs were dissected and treated by either the glyoxilic acid or the paraformaldehyde-glutaraldehyde method. Both methods gave the same stereotypic pattern of stained cells. In the brain lobes, histofluorescence was observed in three bilaterally symmetrical clusters of 4 to 6 neurons each. In the ventral ganglion, a characteristic pattern of fluorescent cells was observed in a row of cells along the dorsal midline and a lateral row of ventrally located cells, consisting of a single cell per hemi-segment in the thoracic and abdominal segments. In addition, a cluster of cells is also present in the subesophageal ganglion. Comparison of the pattern of catecholaminergic neurons in the larval ganglion with serotonergic neurons shows that the two patterns are distinct (see Vallés and White, this volume). CNS of adult and of mutant larvae deficient in the gene *Ddc* were also examined. (Supported by NH grants GM 31503).

273.14 CHARACTERIZATION OF TRANSMITTER INTERACTION AND IMMUNOCYTOCHEMICAL LOCALIZATION OF GAMMA AMINOBUTYRIC ACID AT THE NEUROMUSCULAR JUNCTION OF THE CENTIPEDE Lithobius forficatus. M.E.C. Fitzgerald*†, A.A. Manthey*††, and J.F. Reger*† (SPON: R.B. Caldwell). Division of Neuroscience, Dept. of Anatomy† and Dept. of Physiology††, U.T.C.H.S., Memphis, TN 38163.

Spontaneous depolarizing and hyperpolarizing events were recorded from centipede intersegmental muscle using standard intracellular glass capillary electrodes. These events were somewhat different from miniature end-plate potentials (MEPPs), as recorded from amphibian muscle, in that they had long durations of 100ms or more with slow rise times, and the peak amplitudes of a series of events from the same recording site varied over a wide range from 0.2 to 4.0 mV (mean = 1.10 ± 0.046 (SE) mV) for both types of events. Preliminary histograms suggested quantization of amplitudes of the events.

Local perfusion of bicuculine ($10^{-5}$M, a specific gamma aminobutyric acid (GABA) receptor antagonist) resulted in depolarization and often in contraction of the muscle; in several cases, bicuculine perfusion also eliminated the hyperpolarizing events. In other experiments, perfusion of glutamate di-ethyl ester (GDEE) ($10^{-5}$M, a glutamate receptor antagonist) caused the depolarizing events to disappear. These results suggest that the spontaneous events arise from prejunctional release of GABA and glutamate respectively. Bath application of glutamate ($10^{-5}$M) initially caused depolarization of 5 to 6 mV; following this, fibers spontaneously repolarized despite continued presence of glutamate and after 30 minutes attained values of potentials 5 to 6 mV more negative than resting control values. These results suggest that desensitization of glutamate receptors may be occuring during the repolarization phase and that the hyperpolarization observed is a consequence of continued influence of spontaneous GABA release.

Although these electrophysiologic measurements thus suggest that two transmitter systems are acting on the muscle fibers, prior work (Reger and Fitzgerald, J. Submicrosco. Cytol. 13:1, 1981) had only shown one synaptic morphology. A GABA primary antibody technique was therefore employed to distinguish and localize inhibitory components on the electron microscopic level.

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273.15 CONTRACTIONS OF COCKROACH HYPERNEURAL MUSCLE IN RESPONSE TO GLUTAMATE, PROCTOLIN, OCTOPAMINE, AND A FACTOR FROM THE PINK BOLLWORM. J. I. Moss*, and T. A. Miller* (SPON: A. E. Chalmers). Div. of Toxicology and Physiology, Dept. of Entomology, Univ. of California, Riverside, CA 92521

The cockroach (Periplaneta americana) hyperneural muscle was dissected as described by Miller and James (J. Insect Physiol., 22:981, 1976) and was found to contract in response to a 2.5 µl drop of extract from cockroach abdominal nerve cord, whole pink bollworm (Pectinophora gossypiella) pupae, house fly (Musca domestica) heads and mouse brain. Ion exchange chromatography of these extracts produced three areas of activity. Two of the areas corresponded to the elution volumes of aspartate and glutamate. The third eluted shortly after glutamate.

Of the twenty common amino acids, only glutamate and aspartate caused contraction of this muscle at concentrations at or below $1x10^{-3}$M. Octopamine, applied at $1x10^{-6}$M, caused either no response or a relaxation of the muscle. The pentapeptide, proctolin, caused contractions with a drop on threshold of about $1x10^{-11}$M. Since the third area of activity is stable to acid hydrolysis attempts, it is probably not a peptide. The maximal contractile force elicited by this material is comparable to that of glutamate, although the duration of the response to drop on assay is shorter. The contractions caused by aspartate, glutamate, and the third factor were reversibly blocked by Aranea gemma venom, while the proctolin-induced contractions were not blocked.

273.16 MECHANISMS BY WHICH OCTOPAMINE ENHANCES THE EXCITATORY JUNCTION POTENTIAL AT AN INSECT NEUROMUSCULAR JUNCTION. G. K. Fitch* and A. E. Kammer. Division of Biology, Kansas State University, Manhattan, KS 66506.

Superfusion of Manduca sexta flight muscle with octopamine, a biogenic amine, enhances the amplitude of the excitatory junction potential (EJP) made by the muscle in response to electrical stimulation of its motor neuron. In low-calcium saline, an 18-day-old pharate adult (one day from eclosion) makes a 4 mV EJP in response to motor neuron stimulation. Addition of $5 \times 10^{-6}$ M octopamine to the saline results in a 15 mV EJP. Both the normal and the enhanced EJP reach their peak about 7 ms after the EJP begins, but the enhanced EJP rises at a rate of 3.9 mV/ms whereas the normal EJP rises at a rate of 1.2 mV/ms. One possible explanation for these differences in EJP parameters is that octopamine allows more transmitter to be released from the presynaptic ending, but that the time course of transmitter release is unaffected. However, receptors for octopamine are also present on the postsynaptic cell, since superfusion of $5 \times 10^{-6}$ M octopamine results in a 7 mV hyperpolarization of the resting membrane potential of the muscle cell.

Previous work in our laboratory on 18-day-old pharate adults indicates that synaptic modulation at the neuromuscular junction (NMJ) in these developing moths may be due to both presynaptic and postsynaptic actions of octopamine, since the amplitude and frequency of miniature endplate potentials increases during the application of octopamine. To further investigate possible postsynaptic mechanisms of synaptic enhancement, glutamate, which is known to be a transmitter at some arthropod NMJs, was applied to the preparation in the presence and absence of octopamine. Glutamate alone depolarizes the muscle cell in a dose-dependent manner and is therefore probably the transmitter at this NMJ. In 18-day-old pharate adults, superfusion of $10^{-2}$ M glutamate in 20-second pulses with 15 minutes of saline superfusion between pulses to allow receptor resensitization results in depolarizations of 7 mV. Similar pulses of glutamate in the presence of $10^{-5}$ M octopamine result in 13 mV depolarizations. Thus, postsynaptic actions of octopamine nearly double the response of the muscle to a putative transmitter. Because the response of the muscle to motor nerve stimulation is more than tripled by $5 \times 10^{-6}$ M octopamine, we conclude that octopamine modulation of transmission at this immature synapse involves both presynaptic and postsynaptic mechanisms. Supported by NIH grant NS19257.

273.17 NEUROTOXIC EFFECTS OF 5,6-DIHYDROXYTRYPTAMINE IN THE CENTRAL NERVOUS SYSTEM OF THE FIDDLER CRAB, UCA PUGILATOR. G.K. Kulkarni* and M. Fingerman. Dept. of Biology, Tulane Univ., New Orleans, LA 70118.

5,6-Dihydroxytryptamine (5,6-DHT) is a selective neurotoxin producing degeneration of 5-hydroxytryptaminergic nerve terminals in the central nervous system. 5-hydroxytryptamine (5-HT) appears to function as a neurotransmitter in Uca pugilator. There is strong evidence that 5-HT has the physiological role of stimulating release of some of the hormones, such as the red pigment dispersing hormone, from the neuroendocrine complex in the eyestalk. In two hours a dose of 0.05 ml 5,6-DHT, 0.0025M, per crab induced obvious histomorphological changes in neurosecretory cells in the eyestalks, brain, and thoracic ganglion. With Mallory's triple stain three types of neurosecretory cells are readily distinguished in these structures on the basis of stainability, cell size, nuclear size, and nuclear location. We have, for convenience, designated these cells as types I, II, and III. Only cell types I and II (the two larger types) showed changes after the 5,6-DHT was injected. The size of the type I cells in the eyestalk increased, but decreased in the brain and thoracic ganglion. The nucleus of the type I cells in the brain increased, decreased in the thoracic ganglion, but did not change in the eyestalk. However, the quantity of stored neurosecretory material decreased in the type I cells of all three regions. The size of the type II cells decreased in the three centers, but the size of the nucleus in the type II cells of the brain increased whereas it decreased in the eyestalk and thoracic ganglion. Like the type I cells, the quantity of stored neurosecretory material was reduced in the type II cells of all three regions. These changes in cell types I and II presumably reflect responses to a large degree to depletion of 5-HT in the neurons that are involved in stimulation of the release of the neurohormones they produce. Concomitant with the degeneration of 5-hydroxytryptaminergic neurons that occurs after 5, 6-DHT administration is 5-HT depletion. This depletion would presumably involve release of sufficient 5-HT to stimulate neurosecretory cells that normally respond to 5-HT excitation by releasing their contained neurohormones, thus accounting for the observed decrease in stored neurosecretory material in cell types I and II. The observation that the type III cells showed no changes after the 5,6-DHT was administered is not surprising in view of the fact that in the fiddler crab 5-HT selectively produces hormone release, some hormones being released only by dopamine or norepinephrine. Also, the fact that the type III cells showed no changes is strong indication that the changes in cell types I and II were not merely nonspecific responses to a neurotoxin. Supported by NSF Grant PCM-8300064.

277 SYMPOSIUM. MOLECULAR INSIGHTS INTO GABA-RECEPTOR FUNCTION. J. F. Tallman, Yale Univ. Sch. of Med. and E. Costa, NIMH, St. Elizabeths Hosp., (Chairpersons); C. Braestrup*, Novo Industri, Denmark; H. Möhler*, Hoffmann-LaRoche, Switzerland; J. Bormann*, Max-Plank Institut at Göttingen, FRG; D. Gallager, Yale Univ. Sch. of Med.; A. Guidotti, NIMH.

Recent biochemical, electrophysiological, pharmacological and immunological experiments have provided many details about the molecular structure and function of the GABA receptor complex (Introduction-E.Costa). Current information indicates that this complex is composed of at least two nonidentical subunits and contains binding sites for GABA and drugs which interact with chloride channels. Benzodiazepines are allosteric modifiers of the GABA site and indirectly enhance the actions of GABA. The ability to interact with GABA predicts the relative agonist and antagonist properties of drugs that interact at the benzodiazepine receptor. The target size of the entire complex and individual drug binding sites has been estimated by radiation inactivation (C. Braestrup). The GABA/benzodiazepine receptor complex has been purified by affinity chromatography and monoclonal antibodies against the benzodiazepine receptor have been used to determine details of subunit structure and for immunocytochemical localization (H. Mohler). Multiple conductance states of the GABA receptor have been defined by patch clamp techniques; some of these states are stabilized by drugs (J. Bormann). Altered GABA sensitivity appears to underlie the development of benzodiazepine tolerance; tolerance is rapidly reversed by benzodiazepine antagonist treatment (D. Gallager). The properties and structure of a peptide modulator of benzodiazepine receptors found in brain which possesses anxiogenic activity in a number of behavioral tasks will be described (A. Guidotti). Future molecular approaches to the study of the GABA/benzodiazepine receptor and their implications for human studies will be discussed (J. Tallman).

278 SYMPOSIUM. EFFECTS OF BEHAVIORAL STATE AND MOTOR ACTIVITY ON SENSORY NEURONAL PROCESSING. R. Dubner, NIDR-NIH, and C. Bell, Good Samaritan Hospital (Co-Chairmen); J.C. Houk, Northwestern Univ.; M.C. Bushnell, Université de Montréal, J.M. Miller, Univ. of Michigan; D.L. Robinson, NEI-NIH; J. Fuster, UCLA.

Recent studies indicate that simple and complex behaviors in invertebrates and vertebrates modify the transmission of sensory information at different levels of the central nervous system. This symposium will examine several sensory systems in which signals are modified by motor activity and more complex aspects of behavior involving attention and memory. R. Dubner will introduce the session with an overview of the interaction of behavior and sensory processing. C. Bell will discuss how central discharges in mormyrid fish alter the sensory consequences of motor commands. J.C. Houk will report that the sensitivity of cat inferior olivary cells is attenuated during certain phases of active movement in contrast to sensory responses evoked by passive movement or by external cues. M.C. Bushnell will describe the responses of medullary dorsal horn (trigeminal nucleus caudalis) neurons to innocuous and noxious thermal stimuli in monkeys trained in a detection task. She will show how attentional factors modify the monkey's ability to detect these stimuli. J.M. Miller will report on studies of neurons at various levels in the monkey auditory system, including the cerebral cortex, and how behavioral tasks and training can dramatically alter cellular responses. D.L. Robinson will describe the visual properties of neurons in the pulvinar thalamic nuclei of the primate during behavior. He will show how responses during specific tasks are modified by changes in neurotransmitter activity and are related to attention. J. Fuster will show that neurons in the inferotemporal and frontal cortices are responsive to visual stimuli and that their discharges can be modified when a monkey performs a visual memory task. A discussion period will follow in which the participants will examine possible directions for future research in this area.

## TROPHIC INTERACTIONS I

279.1 THE EFFECT OF TRANSSYNAPTIC STIMULATION ON THE MORPHOGENESIS OF THE AMPHIBIAN MAUTHNER CELL. L.A. Goodman and P.G.Model. Dept.Neurosci., Albert Einstein Coll. Med., Bronx, NY 10461

Afferent innervation affects the properties of target neurons. During development, formation of synaptic contacts may be essential for the normal differentiation and maintenance of postsynaptic neurons. We have examined the extent to which ingrowing axons regulate the morphogenesis of a single target neuron, the Mauthner cell (M-cell). M-cells occur as a pair of large, uniquely identifiable neurons in the medulla of fish and premetamorphic amphibians. In the axolotl (Ambystoma mexicanum), M-cells are located at the level of entry of the vestibular nerve (nVIII); each receives synapses from the ipsilateral nerve as a major source of afferent input. nVIII terminals are morphologically distinctive as club endings in both the light and electron microscopes. These endings are restricted to the proximal ventral surface and branches of the M-cell lateral dendrite. Here we report on the effects of superinnervation on the growth and branching of this portion of the M-cell lateral dendrite.

Superinnervation was brought about by the unilateral implantation of an extra vestibular primordium (together with its associated nVIII ganglion) rostral to the in situ one. Surgery was performed prior to nVIII ougrowth and M-cell differentiation. The contralateral side served as a control. When the animals reached the 21mm larval stage, the ectopic nerve was labelled with horseradish peroxidase (HRP).

Subsequent LM examination of the larvae showed that both the ectopic and in situ vestibular primordia had developed into anatomically normal ears. HRP-labelled vestibular axons entered the brain at the level of nV and coursed caudad in the nVIII tract. EM analysis demonstrated labelled club endings on the appropriate region of the experimental M-cell lateral dendrite. Comparison of LM-reconstructed experimental and control M-cells revealed that superinnervated M-cells had significantly enhanced dendritic branching in the region of the lateral dendrite that normally receives vestibular input. No changes were observed in the size and shape of the M-cell soma, medial dendrite, or axon.

These results support the hypothesis that afferent input has a transsynaptic trophic effect on the morphogenesis of target neurons. Our data, together with the results of deprivation experiments (Kimmel, Develop. Biol., 55:244-259, 1977), indicate that ingrowing axons have an essential role in regulating the elaboration of a normal dendritic branching pattern by target neurons during development.

(Supported by NIH grants 5T32GM7288 and NS 18823)

279.2 PRESYNAPTIC INFLUENCE ON POSTSYNAPTIC CHANNEL KINETICS IN FROG SYMPATHETIC NEURONS. L.M. Marshall* (SPON: D.E. Millhorn). Dept. of Physiol., Univ. of North Carolina, Chapel Hill, NC 27514

Sympathetic ganglia of the frog contain two types of principal neurons, B and C neurons, which are selectively innervated by two different classes of cholinergic preganglionic axons, B and C fibers. In both types of ganglionic neurons, the fast excitatory postsynaptic currents (EPSCs) are mediated by nicotinic ACh-gated channels. This study examined the EPSCs and ACh-gated channels of B and C neurons in normal ganglia and in ganglia where denervated B neurons were incorrectly innervated by preganglionic C fibers. The results provide evidence that the kinetic properties of postsynaptic ACh-gated channels depend on the particular class of innervating preganglionic axon.

Neurons in the 9th and 10th lumbar sympathetic ganglia of adult bullfrogs were identified according to conduction velocity and voltage clamped at -50 mV (20-22°C). In normal ganglia, the time constant for the exponential decay of the EPSCs was 5.5 ±1.1 msec for B neurons and 10.2 ±2.3 msec for C neurons (mean ±S.D.). Spectral analysis of ACh noise revealed that these different EPSC decay rates correspond to similar differences in the mean lifetime of ACh-gated channels of the B neurons (5.2 ±0.9 msec; ±S.D.) and C neurons (9.8 ±2.0 msec). This indicates that the EPSC decay is governed by the rate of channel closure.

To innervate B cells with C fibers, preganglionic B fibers were cut while the C fibers remained intact. Three weeks after surgery, B neurons were identified by antidromic conduction velocity (> 1 m/sec) and found to be innervated solely by preganglionic C fibers. These incorrectly innervated B neurons had slowly decaying EPSCs and long mean channel open times which were indistinguishable from those of normal C neurons.

B neurons maintained these slower ACh-gated channels for over three months when the surgery was repeated to prevent the return of the preganglionic B fibers. When B neurons were reinnervated by B fibers, the mean EPSC decay time was not significantly different from that of B neurons in normal ganglia. This indicates that the expression of slower ACh-gated channel kinetics in B neurons is not a consequence of reinnervation alone, but a specific response to innervation by preganglionic C fibers. (Supported by grants NS 17203 & NS 14899.)

279.3 MUSCLE FIBERS ARE REQUIRED FOR THE SELECTIVE ACCUMULATION OF CONNECTIVE TISSUE CELLS IN JUNCTIONAL REGIONS OF DENERVATED SKELETAL MUSCLES. Elizabeth A. Connor and U.J. McMahan. Dept of Neurobiology, Stanford University, Stanford, CA 94305.

Denervation of a skeletal muscle results in a selective accumulation of connective tissue cells in the former region of innervation. These connective tissue cells are structurally similar to fibroblasts. Such cells may play a crucial role in reestablishing neuromuscular function after nerve damage; connective tissue cells make and degrade extracellular matrix, and the extracellular matrix of nerve and muscle is directly involved in regeneration of the neuromuscular junction.

We have undertaken a series of studies aimed at determining the function of the connective tissue cell aggregates and learning how the accumulation of the cells is regulated. In the experiment described here we examined the role of components of the denervated neuromuscular junction--the muscle fiber, degenerating axon terminal and the Schwann cell associated with the terminal--in directing the accumulation of connective tissue cells. We damaged frog muscles in ways that caused degeneration of the muscle fibers but left intact myofiber basal lamina, the axons that innervated the muscle fibers and the Schwann cells. Regeneration of muscle fibers was prevented. One month later, when all of the original muscle fibers had been phagocytized, the axons were damaged. In the absence of muscle fibers, but in the presence of degenerating axons and Schwann cells, there was no accumulation of the connective tissue cells in the former junctional region. The usual accumulation was observed in denervated intact frog muscles and damaged frog muscles where the muscle fibers were allowed to regenerate. We conclude that neither degenerating axons nor denervated Schwann cells are sufficient to generate the connective tissue cell response to muscle denervation; on the other hand a signal provided by myofibers is required for the accumulation of cells to occur. Supported by an MDA Postdoctoral Fellowship to E.A.C. and NIH grant NS 14506.

279.4 FACTORS THAT INFLUENCE THE MAINTENANCE OF REGENERATING AXON TERMINALS AT NEUROMUSCULAR JUNCTIONS. Y.M. Yao & U.J. McMahan, Dept. of Neurobiology, Stanford Univ. Sch. Med., Stanford, Ca. 94305.

The maintenance of motor axon terminals at the neuromuscular junction in skeletal muscles of the adult frog is virtually independent of muscle fibers. Accordingly, if muscles are damaged in ways that result in degeneration and phagocytosis of muscle fibers but spare the muscle fiber basal lamina sheaths and motor axons, the axon terminals persist at synaptic sites on the sheaths for more than a year (Yao & McMahan, Soc. Neurosci. Abs., 10, 1085, 1984). On the other hand, regenerating axon terminals are highly dependent on muscle fibers for their maintenance. If axons as well as muscle fibers are damaged, the axons regrow to the original synaptic sites on the empty muscle fiber basal lamina sheaths but most terminals disappear from the synaptic sites within a few months (Sanes et al., J.C.B. 78: 1978, Yao & McMahan, Soc. Neurosci. Abs., 10, 1085, 1984).

In the study presented here, we sought to determine whether regenerating axons acquire the ability to persist in the absence of muscle fibers if they first reinnervate the muscle fibers. We crushed the nerve to the frog's cutaneous pectoris muscle. Seven to ten days after the axons had reinnervated the muscle fibers, we caused muscle fiber degeneration without damaging the axon terminals. Five months later, 65% of the synaptic sites on the empty basal lamina sheaths were still occupied by regenerating axon terminals. On the other hand, in muscles 5 months after reinnervation of empty basal lamina sheaths only 5% of the synaptic sites were occupied by axon terminals. We conclude that adult muscle fibers provide a factor or factors that enable regenerating axon terminals to persist without the muscle fibers and that this long-term stabilization effect is achieved within a few days after reinnervation.

Supported by a NRSA fellowship NS 07089 and a National Amyotrophic Lateral Sclerosis postdoctoral fellowship to Y.M.Y. and NIH grant NS 14506.

279.5 CELL MEMBRANE CONTACT REGULATES TRANSMITTER PHENOTYPIC EXPRESSION IN CULTURED SYMPATHETIC NEURONS. J.E. Adler and I.B. Black. Division of Developmental Neurology, Cornell Univ. Med. Coll., N.Y., NY 10021.

We have previously reported that high neuronal density with attendant aggregation, selectively increases expression of choline acetyltransferase (CAT) and substance P (SP) in dissociated, pure neuronal cultures of rat superior cervical sympathetic ganglia. To investigate the specific role of cell contact in selective transmitter expression, CAT activity and SP content in high density cultures were examined under various circumstances. CAT activity appeared de novo after two days in culture and rose rapidly thereafter. Similarly, SP content, detectable 6 hours after plating, doubled during the first two culture days and subsequently increased more than 10-fold. The increases closely paralleled perikaryal aggregation, suggesting that cell contact may be a critical factor. Moreover, interference with aggregation physically, by methylcellulose, or chemically, by tunicamycin, inhibited the increases in CAT activity and SP content without affecting neuronal survival. Thus, cell contact appeared to mediate the expression of CAT and rise of SP in high density neuronal cultures.

To elucidate potential molecular mechanisms and to determine whether interaction of membrane component(s) elicited the rises in CAT activity and SP content, ganglion cell membranes were added to cultures of varying densities. After three days in high density cultures, cell membranes doubled the increases in CAT and SP. Moreover, even in lower density cultures, membranes elicited the appearance of CAT activity. Our studies suggest that interaction of cell membrane components regulate phenotypic expression in aggregating neurons. We are currently defining the characteristics of this membrane factor. (Supported by NIH Grants NS 10259 and HD 12108).

279.6 THE TARGET HIPPOCAMPUS INCREASES TYROSINE HYDROXYLASE AND NE UPTAKE IN LOCUS COERULEUS IN CULTURE. C.F. Dreyfus, M.A. Gillies*, M. Goldstein and I.B. Black. Div. of Developmental Neurology, Cornell Med. Coll. New York, NY 10021 and Dept. of Psychiatry, New York Univ. Med. Center, New York, NY 10016.

To define mechanisms underlying brain development, we have been examining maturation of noradrenergic traits in the mouse locus coeruleus (l.c.) in vivo and in culture. Previous studies indicated that tyrosine hydroxylase (TH) and the specific, high-affinity norepinephrine (NE) uptake system exhibit developmental increases in culture, reflecting ontogeny in vivo. Further, depolarizing agents significantly increase TH in l.c. neurons without affecting NE uptake in the same cells, suggesting that two noradrenergic characters exhibited by the same neuron may be independently regulated.

To determine whether locus cells are similarly affected by another extracellular signal, we now examine the effects of a normal locus target, the hippocampus, on maturation of TH and NE uptake.

Locus explants were dissected from mice at embryonic day 14 (E-14) and co-cultured with either the E-18 iris or E-18 hippocampus. The iris was used as an inappropriate target control. Within 7 days, the locus elaborated TH-containing neurites which contacted the co-cultured iris or hippocampus. After 5 weeks, hippocampal-locus co-cultures exhibited a significant increase in both TH activity and NE uptake, in striking contrast to iris-locus controls. Consequently, the hippocampal target apparently influenced these two locus traits similarly.

In conclusion, our studies suggest that the specific hippocampal target regulates maturation of two NE traits associated with the same locus neurons. We are now in position to define molecular mechanisms underlying effects of extracellular stimuli on critical transmitter traits in brain cells in culture. (Supported by NIH Grant NS 20788, NSF Grant BNS80-24081 and the American Parkinson Disease Association. CFD is the recipient of a Teacher-Scientist Award from the Andrew W. Mellon Fdn.).

279.7 TROPHIC EFFECTS AND SURVIVAL OF PC12 CELL GRAFTS IN RAT BRAINS. C.B. Jaeger. Department of Physiology & Biophysics, New York University Medical Center, New York, NY 10016.

Neural transplants grown in the brain are useful for the study of numerous questions concerning neural development, plasticity, trophic influences, regeneration, and biosynthetic prostheses. One drawback of neural grafts is that they have a heterogeneous composition of various neuronal and non-neuronal cell types. To circumvent this problem the present study used grafts consisting of one cell type only, namely PC12 cells. PC12 pheochromocytoma cells may express some neuron-like characteristics such as process extension when exposed to nerve growth factor and fibroblast growth factor. Moreover, PC12 cells synthesize catecholamines, such as dopamine, and PC12 cells therefore could provide a possible biosynthetic dopamine replacement source. However, previous work showed rejection of PC12 cells when they were transplanted subcutaneously to a species other than New England Deaconess Hospital rats from which PC12 cells were originally derived.

In this study variable numbers of cultured PC12 cells were aggregated and implanted into one of the following regions of the brain: lateral ventricles, tectum, cerebral cortex, ventral forebrain. The hosts were Sprague Dawley (SD )rats ranging in age from four days to six weeks. Following sacrifice of the hosts between two weeks and two months post-transplantation, the brain tissues containing the PC12 cell graft were histologically processed for localization of enzymes and glia filaments. It was found that PC12 cell aggregate grafts survive transplantation to the brain of either immature or mature SD rats. Graft volume inceased over time as a result of continued cell division. Implanted PC12 cells exhibited immunoreactivity to antisera against tyrosine hydroxylase and aromatic amino acid decarboxylase but not phenylethanolamine n-methyltransferase, suggesting dopamine synthesis by the grafted PC12 cells. Adjacent to large fiber tracts and in deep layers of the host cortex, some of the PC12 cells grew processes. PC12 cell grafts were extensively vascularized by host-derived cerebral microvessels. Observation of the ultrastructure revealed the formation of fenestrated capillaries in PC12 cell grafts a finding which was confirmed by leakieness of the "blood graft barrier" to circulating horseradish peroxidase and Evans Blue. Grafts that were four to eight weeks old contained migrated fibrous astrocytes and in these cases the host brain bordering the PC12 cell grafts was populated by notably reactive astroglia .

The observed structural alterations of host-derived cells and some grafted PC12 cells suggest the presence of bi-directional trophic interactions between the host brain and the PC12 cells.
(Supported by USPHS Grant NS-19699 from NINCDS).

279.8 CORONARY PERFUSATE OF THE RAT, GUINEA PIG AND RABBIT SUPPORTS THE SURVIVAL OF EMBRYONIC SYMPATHETIC NEURONS IN CULTURE. Arun R.Wakade, John C. Prat* and Taruna D. Wakade*. Department of Pharmacology, State University of New York Downstate Med. Ctr., Brooklyn, NY 11203

Sympathetic neurons (SN) derived from peripheral ganglia of the chick embryo continue to survive and grow in culture supplemented with essential neurotrophic factors. For example, nerve growth factor (NGF) or a medium conditioned by chick embryonic heart cells is known to support the survival and induce neurite outgrowth of SN in culture (Edgar et al., Nature 289: 294, 1981). We report here another source which provides a rich supply of material that exerts a powerful effect on the survival and neurite outgrowth of SN maintained in culture.

SN were derived from 10- to 12-day-old chick embryonic paravertebral and lumbo-sacral sympathetic ganglia and plated on polyornithine-coated dishes containing F14 culture medium. Hearts obtained from adult male or female rats, guinea pigs or rabbits were perfused through aorta (Langendorf method) with a culture medium (F14) at a rate of about 2 to 7 ml/min at 37°C. The perfusate was sterilized by filtration and added to culture dishes along with insuline and transferrin (1 µg/ml each) (Wakade et al., Exp. Cell Res. 140: 79, 1982). Within minutes after the addition of the heart perfusate (HP), neurons began to show the outgrowth of neurites with prominent growth cones. After 1 to 2 hr several neurons had neurites of 2 to 3 times the size of their cell body. About 60% of the plated cells survived in culture up to 2 to 4 days in the presence of HP. These effects occurred in the absence of NGF. However, addition of NGF exaggerated the above effects, and essentially all the plated cells survived in the HP. SN surviving in the presence of HP exhibited intense neurite outgrowth, and the cell bodies appeared to be enlarged. The culture substrate treated with HP for 2 hr and then washed 2 times with saline supported the survival of neuronal cells for 2 days, even in the absence of NGF. Secondly, if the cultures were initiated first in normal culture medium containing NGF and after 2 days the medium was changed to HP plus NGF, it was found that the cells took up more $^{3}$H-norepinephrine and contained more ATP than those cells that remained in normal medium plus NGF throughout. Perfusates of rat kidney, salivary gland and the adrenal gland did not show neuron-supporting activity. Removal of both auricles, loss of cardiac activity (rate and force of contraction) and removal of $Ca^{++}$ from the perfusion medium did not abolish the survival effect of the HP.

It is concluded that adult mammalian cardiac muscle releases substances in the circulation which markedly influence the survival and morphological differentiation of SN.

(Supported by NIH Grant No. HL18601 and NSF Grant No. BNS8409685.)

279.9 PROLONGED PRODUCTION OF NEURITE PROMOTING ACTIVITY AFTER PERMANENT NERVE TRANSECTION. A.J. Windebank and J.F. Poduslo. Peripheral Nerve Center, Mayo Clinic and Mayo Foundation, Rochester, MN 55905.

Cells from previously transected rat sciatic nerve support neurite outgrowth from embryonic rat dorsal root ganglia (DRG) (Richardson and Ebendal, Brain Res. 246:57-64, 1982; Windebank and Poduslo, Neurol. 35:294, 1985). We now demonstrate that this activity depends on time of explantation after permanent transection and is not due to NGF activity.

Sciatic nerves were permanently transected (Poduslo et al, J. Neurochem. 44:388-400, 1985) and then endoneurial tissue explanted onto collagen-coated dishes at various times. After 24 hours in culture, E15 rat DRG were added either as co-culture or separately with or without NGF. Neurite outgrowth from DRG was measured on photomicrographs of replicate cultures (n=8 to 42) after 24, 48, and 90 hours. Outgrowth was linear during this period.

| Culture Conditions | Neurite outgrowth from DRG at 90 hours mm(Mean ± S.E.M.) | Significance of difference from control |
|---|---|---|
| NGF 100 mg/ml | 1.30 ± .05 | |
| No NGF | 0 ± 0 | |
| Co-culture (days post transection) | | |
| 0 | 1.16 ± .08 | NS |
| 2 | 1.12 ± .07 | NS |
| 5 | 1.00 ± .06 | < .005 |
| 7 | 1.02 ± .05 | < .025 |
| 14 | 0.57 ± .09 | < .001 |
| 35 | 0.99 ± .08 | < .01 |
| 41 | 1.33 ± .05 | NS |

This suggests a biphasic production of neurite promoting activity.

The effect was not inhibited at 7, 14, and 35 days by two anti-NGF polyclonal sera. Although the possibility of two different factors is raised by these observations, their chemical nature, the specific cells producing the factor(s), and possible subpopulations of responsive DRG neurons, are still to be identified.

279.10 CHARACTERIZATION OF THE BINDING PROPERTIES AND RETROGRADE AXONAL TRANSPORT OF A MONOCLONAL ANTIBODY DIRECTED AGAINST THE RAT NERVE GROWTH FACTOR RECEPTOR.
M.Taniuchi* and E.M.Johnson, Jr. (SPON: D.B. McDougal,Jr.) Dept. of Pharmacology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

We have demonstrated in vitro and in vivo the specific binding of a monoclonal antibody to the rat nerve growth factor (NGF) receptor. Previous work had shown that this antibody (designated "192-IgG"), a product of the immunization of BALB/c mice with solubilized plasma membrane proteins of PC12 cells, does not compete with NGF for binding to the NGF receptor of PC12 cells, but instead interacts with the receptor to increase NGF binding to these cells (Chandler, C.E., L.M. Parsons, M. Hosang, and E.M. Shooter, 1984, J. Biol. Chem., 259: 6882-6889). In the present study, a solid-phase separation assay verified the specific formation of a ternary complex of 192-IgG, the NGF receptor, and NGF: $^{125}$I-labeled 192-IgG precipitated from solution only when incubated with both solubilized NGF receptor and NGF covalently linked to a solid phase (Sepharose 4B). The 192-IgG also mediated the specific precipitation of NGF receptor molecules which were either covalently coupled to $^{125}$I-labeled NGF or directly radioiodinated. Filtration assays using plasma membrane preparations of various rat tissues showed strict correlation of $^{125}$I-192-IgG and $^{125}$I-NGF binding; only membranes obtained from superior cervical ganglion (SCG) bound significant amounts of the monoclonal antibody and NGF.

Injection of $^{125}$I-192-IgG into the rat anterior eye chamber led to a specific accumulation of intact antibody molecules (as assessed by SDS-polyacrylamide gel electrophoresis) in the ipsilateral SCG, evincing the retrograde axonal transport of the monoclonal antibody from the neuronal termini, located at the iris, to the cell bodies situated in the ganglion. The time course and saturation characteristics of $^{125}$I-192-IgG retrograde transport were very similar to those previously reported for $^{125}$I-NGF transport, indicating that 192-IgG can be internalized and transported by the same mechanisms as is NGF, and suggesting that the NGF receptor itself is also retrogradely transported. Consistent with results of the in vitro binding assays, 192-IgG and NGF failed to compete for retrograde transport and were actually co-transported. Retrograde axonal transport of 192-IgG appears to be species-specific, since $^{125}$I-192-IgG was transported in the rat, but not in mice, gerbils, hamsters, or guinea pigs. These results establish monoclonal antibody 192-IgG as a specific probe for the rat NGF receptor in vitro and in vivo.
(Supported by a grant from the Monsanto Company and by NIH training grant 5-T32-GM07805).

279.11 SELECTIVE DESTRUCTION OF NGF RECEPTOR-BEARING CELLS USING A HYBRID TOXIN. P.S. DiStefano, J.B. Schweitzer and E.M. Johnson, Jr. Dept. of Pharmacology, Washington Univ. Sch. of Med., St. Louis, Mo. 63110.

A hybrid toxin composed of ricin A chain and a monoclonal antibody directed against the rat NGF receptor (IgG 192) was prepared using the heterobifunctional cross-linking agent N-succinimidyl-pyridyldithio-propionate (SPDP) and purified by affinity chromatography. Characterization studies showed that the hybrid, 192-s-s-A, displaced bound $^{125}$I-192 from rat superior cervical ganglion (SCG) membranes with an IC 50 3-5 times lower than that of unconjugated 192. When incubated with cultured rat SCG neurons 192-s-s-A inhibited protein synthesis in a concentration-dependent fashion. The effect of 192-s-s-A on these neurons was reversed by coincubation with an excess of 192. The IC 50 of 192-s-s-A on protein synthesis in rat SCG neurons was 4 nM. Intact ricin and ricin A chain inhibited protein synthesis in these neurons with IC 50 values of 5 pM and 500 nM, respectively. The 192-s-s-A hybrid had no effect on mouse SCG neurons or a human melanoma cell line known to have NGF receptors. This is consistent with the ability of 192 to recognize only the rat NGF receptor. Also, 192-s-s-A did not inhibit protein synthesis in primary rat muscle cell cultures or Vero cells, which do not have cell surface receptors for NGF. 192-s-s-A was able to inhibit protein synthesis in PC12 cells but the potency was 10-100 times less in these cells compared to rat SCG neurons. Ricin and A chain were also 10-100 times less potent in PC12 cells than neurons. Rat SCG neurons exposed to 192-s-s-A lost their refractile appearance under phase optics, showed granular degeneration of neurites, and died. Thus the decreased protein synthesis caused by the hybrid toxin correlated with the morphological destruction of the neurons.

Studies in vivo indicated that $^{125}$I 192-s-s-A was retrogradely transported from the iris muscle to the superior cervical ganglion (SCG) in the rat. Transport of hybrid was blocked by an excess of 192, but not A chain. Gel electrophoresis and subsequent autoradiography of SCG extracts indicated that free A chain, as well as intact 192-s-s-A and 192, was present in the SCG. Similar results were seen in the rat dorsal root ganglion when $^{125}$I 192-s-s-A was injected into the crushed sciatic nerve. These results indicate that ricin A chain can be delivered to the cell bodies of NGF receptor-bearing cells.

192-s-s-A represents a potentially powerful tool by which to selectively destroy NGF receptor-bearing cells in vitro. The hybrid toxin may prove useful as an in vivo toxin. (Supported by a grant from the Monsanto Company).

279.12 THE USE OF MONOCLONAL ANTIBODIES DIRECTED AGAINST THE GANGLIOSIDE GM1 TO PROBE NGF-MEDIATED NEURITOGENESIS OF EMBRYONIC CHICK SENSORY GANGLIA IN VITRO. S.G. Matta*, M.M. Rapport and F.J. Roisen. Dept. of Anatomy, UMDNJ-Rutgers Medical School, Piscataway, NJ 08854 and Div. of Neuroscience, New York State Psychiatric Institute, NY, NY 10032.

Gangliosides are acidic glycosphingolipids that are prominent components of neuronal membranes and may play a major role in cell surface functions. In several neuronal cell models, an interaction between the ganglioside GM1 and nerve growth factor (NGF) has been shown to exist. To investigate the specific nature of the NGF-ganglioside interactions, we have employed five different monoclonal antibodies (mAbs) directed against GM1 (designated B6, C3, C4h2, D1 and D3) to probe NGF-mediated neuritogenesis. The survival and differentiation of embryonic chick sensory ganglia in vitro (dorsal root ganglia, DRG) are NGF-dependent. Our previous work and that of others have shown that exogenous bovine brain gangliosides potentiate these responses. The separate growth parameters of DRG studied were: 1) neurite induction, quantitated by computer-assisted measurement of neurite length and number per explant and 2) general metabolic activity, assayed by measuring levels of ornithine decarboxylase (ODC), the rate-limiting enzyme in polyamine biosynthesis. For morphologic evaluation, DRGs were dissected from 9 d embryonic White Leghorn chicks and grown for 44 h in Medium 199 + 10% FBS and 10 mg/dl gentamicin, supplemented with 10 ng/ml 2.5s NGF and 20% ascites fluid containing one of the five mAbs to GM1. Control groups were exposed to medium containing 20% ascites fluid without mAb. Image-analysis revealed that the mAbs did not produce equivalent responses. C4h2, D1 and D3 inhibited NGF-stimulated neurite length and number, whereas B6 and C3 reduced only number. Metabolic activity was assessed by incubating DRGs for 5 h in serum-free medium, supplemented with 20% mAb ascites fluid, in the presence or absence of 10 ug/ml 7s NGF. These studies demonstrated further that only mAb B6 and C4h2 inhibited the NGF-stimulated elevation of ODC. Comparison of the individual effects of the mAb on neurite length, number and ODC activity revealed that each elicited a specific response. Both B6 and C3 mAbs reduced neurite number but not length, while only B6 inhibited the NGF-mediated ODC elevation. mAb D1 resulted in the greatest reduction in neurite length and number, but did not alter ODC levels. These studies have shown that the ganglioside GM1 regulates NGF-mediated neuronal development and suggest that individual sites on the ganglioside molecule may modulate specific neurotrophic interactions. Supported by NIH NS11299.

279.13 CORRELATION BETWEEN LONG-TERM $GM_1$ GANGLIOSIDE EFFECTS ON CNS REPAIR AND NEUROTROPHIC ACTIVITY. A. Consolazione*, C. Aldinio*, D. Presti*, R. Dal Toso*, A. Leon and G. Toffano (SPON: G. Calderini). Fidia Neurobiological Research Laboratories, Abano Terme, Italy

We have already documented that chronic $GM_1$ monosialoganglioside treatment facilitates the repair of the nigro-striatal pathway in adult rats following unilateral hemitransection but not following a single unilateral intranigral injection of 6-OH-DA (Toffano, G., et al., Acta Physiol. Scand., 122:313, 1984). In this context we have recently provided evidences indicating that the lack of $GM_1$ effects in the 6-OH-DA lesioned animals may be related to differences in lesion-induced neuronotrophic (NTF) activity. Therefore it has been proposed that $GM_1$ may necessitate adequate titers of NTF induction so as to facilitate CNS repair processes (Toffano, G., et al., Soc. for Neurosci. Ann. Meeting, abst. 194.2, 1984).

As induction of neuronotrophic activity following injury has been reported to be slower in aged rats than in young adults (Needles, D.L., et al., Soc. for Neurosci. Ann. Meeting, abs. 243.14, 1983), we investigated the capability of $GM_1$ to facilitate repair in hemitransected rats of different ages. Results obtained indicate that $GM_1$ is capable of enhancing CNS repair in rats of all ages considered (2-18 months). Nevertheless, the $GM_1$ treatment protocol, necessary to obtain long-term beneficial effects in hemitransected rats, is dependent on the age of the rats, i.e. with increasing age there occurs an increasing latency in the appearance of $GM_1$ effects on striatal tyrosine-hydroxylase (TH) activity. These results further support the hypothesis that NTF molecules, although still unidentified, may indeed be essential for long-term $GM_1$ effects in vivo as in vitro (Ferrari, et al., Dev. Brain. Res., 8:215, 1983). Along this line, experiments are in progress to assay and characterize the neuronotrophic activity in the striatum and substantia nigra of hemitransected rats of different ages.

279.14 PURIFICATION AND CHARACTERIZATION OF NEURONOTROPHIC ACTIVITY FROM BOVINE CAUDATE NUCLEI: POSSIBLE MODULATION BY $GM_1$ GANGLIOSIDE. R. Dal Toso*, O. Giorgi, D. Presti*, M. Favaron*, A. Leon and G. Toffano. Fidia Neurobiological Research Laboratories, Abano Terme, Italy.

Specific extrinsically occurring neuronotrophic factors (NTF) are known to regulate cell survival and neurite outgrowth of embryonic neurons both in vivo and in vitro. Recent evidences suggest that similar or functionally equivalent activities may be operative in the adult CNS not only for the maintenance of neuronal survival and specific synaptic connections but also for the occurrence of functional neuronal repair following damage, (Nieto-Sampedro, M., et al., J. Neurosci., 3:2219, 1983). In this context we have provided evidences suggesting that long-term $GM_1$ beneficial effects on CNS repair may necessitate NTF induction and possibly occur via modulation of NTF activity in vivo as in vitro (Toffano, G., et al., Soc. for Neurosci. Ann. Meeting, abst. 194.2, 1984). To verify these hypotheses, attempts were made to detect and purify NTF(s) from extracts of the bovine caudate nuclei.

Serumless dissociated fetal mouse mesencephalic cell cultures were utilized as bioassay and the NTF activity quantified by measuring specific $^{3}$H-dopamine uptake, specific $^{14}$C-GABA uptake and neuronal cell survival with time in culture. Data obtained indicate that crude supernatant preparations (100,000 x g for 2 hrs) contain activity capable of increasing all parameters considered. Purification procedures, including Sephadex G-150 gel filtration and HPLC cationic exchange chromatography, indicate that the NTF activity is associated with a non-dializable, trypsin and heat-labile basic molecule(s). This activity is presumably not nerve growth factor (NGF) since NGF, when applied to mesencephalic cell cultures, does not modify any of the parameters considered and NGF antiserum does not decrease the extracted NTF activity.

Preliminary results indicate that similar activity occurs in crude striatal extracts from rat brain. The titers of such activity increase following injury and its effect on CNS cultured neurons is amplified by $GM_1$. Further purification and biological characterization of the reported NTF(s) is currently in progress.

280.1 REDUCTION IN 2-DEOXY-D-GLUCOSE AND COLD-WATER SWIM ANALGESIA IN AGED RATS. E. Kramer and R.J. Bodnar, Dept. of Psychology, Queens College, CUNY, Flushing, NY 11367.

Compromised coping responses, including impairments in glucose tolerance and thermoregulation occur during aging. Since acute exposure to either 2-deoxy-D-glucose (2DG) or cold-water swims (CWS) produces analgesia in young adult rats, the present study examined whether alterations in these responses occurred as a function of age. Further, to determine the relative specificity of any observed changes, aging effects upon 2DG hyperphagia and CWS hypothermia were also examined. First, separate cohorts of 4, 9, 14, 19 and 24-month old female rats received ascending doses of 2DG (0, 50, 250, 450 and 650 mg/kg, IP) with tail-flick latencies and jump thresholds assessed 30, 60 and 120 min later. Then the same rats received 2DG injections (0, 650 and 1200 mg/kg) and food intake was assessed 5 h later. Significant decreases in 2DG analgesia were observed on both the tail-flick and jump tests as a function of age with the maximal decrease observed at the highest 2DG dose. The magnitude of the effect was more robust on the tail-flick test. 2DG hyperphagia systematically decreased with age: significant hyperphagia was noted in the 4 and 9-month groups, no change in intake was noted in the 14-month group, and significant hypophagia was noted in the 19 and 24-month groups. Second, new cohorts of rats received a no swim control and a 3.5 min CWS at a water temperature of 2°C with tail-flick latencies, jump thresholds and core body temperatures assessed 30, 60 and 90 min later. CWS analgesia was eliminated in the 24-month group on both pain tests, and was attenuated in the 19-month group on the jump test. The reductions in CWS analgesia could not be attributed to concomitant changes in hypothermia since CWS hypothermia was actually more pronounced in the three older cohorts. While the decrements in 2DG analgesia appear progressively through adulthood, the decrements in CWS analgesia appear more abruptly in the aged animal. (Supported by NIH Grant AG-04425).

280.2 EFFECTS OF AGE AND GENDER ON POSTMORTEM AMINE RECEPTOR BINDING TO HUMAN FRONTAL CORTEX. J.J. Mann, P.A. McBride*, M. Stanley* and C. Petito*. Psychopharmacology Laboratory, Department of Psychiatry, Cornell University Medical College, New York, N.Y. 10021.

Amine receptor binding and monoamine oxidase (MAO) A and B kinetics were assayed in postmortem samples of human frontal cortex and head of caudate nucleus. A statistically significant positive correlation was found between age and MAO B ($r=0.6$, $p<0.01$), but not MAO A activity (Vmax). A negative correlation was found between age and the number of serotonin-2 ($5\text{-HT}_2$) binding sites in frontal cortex ($r=-0.42$, $p<0.01$). A positive correlation was found between age and beta adrenergic binding in frontal cortex ($r=0.6$, $p<0.01$). No significant age effects were seen on $5\text{-HT}_1$ receptor binding to frontal cortex or $^3$H-spiroperidol (dopamine$_2$) binding to caudate nucleus. No significant correlation with postmortem delay was found for any of these amine receptor and enzyme measures. There were no gender differences in receptor binding measures or MAO enzyme kinetics. Assay of amine receptor binding in postmortem human brain tissue appears to be a potentially valuable strategy for studying aminergic systems in psychiatric and neurological disorders when specific effects of age are taken into account. This work was supported by PHS grant #MH 40210 to Dr. Mann.

280.3 STUDIES ON CATECHOLAMINE FUNCTION AND AGING: IN VITRO UPTAKE AND EFFECTS OF COLD STRESS. H. McIntosh and T.C. Westfall. Department of Pharmacology, St. Louis University School of Medicine, St. Louis, MO 63104.

We have continued our systematic study of adrenergic function during the aging process. Present experiments have been carried out utilizing Fischer 344 rats at 2-4 months, 11-13 months, and 21-25 months to assess the effect of cold stress on urinary norepinephrine (NE) levels and in vivo tyrosine hydroxylase activity as well as to assess in vitro uptake.

For urinary NE measurements, test rats were maintained 48 hours at 4-6°C in metabolic cages after acclimating to the cages for 48 hours at 22-24°C. Control rats were kept in metabolic cages for 96 hours at 22-24°C. Urine was collected in 1 N acetic acid for the last three 24 hour periods in the cages. NE was isolated using alumina, and then measured using HPLC with electrochemical detection. There was an age related attenuation of the increase in urinary NE in response to cold stress.

One month later, in order to evaluate in vivo tyrosine hydroxylase activity, the rats were again acclimated to the metabolic cages. Then test rats were placed in precooled metabolic cages at 4-6°C for one hour prior to sacrifice. After 30 minutes in the cages, rats were injected i.p. with m-hydroxybenzylhydrazine (100 mg/kg). Control rats were injected similarly 30 minutes prior to sacrifice. Hypothalamus, striatum, and occipital cortex were dissected out and frozen immediately on dry ice. The tissue was later analyzed for dihydroxyphenylalanine (DOPA) accumulation using HPLC with electrochemical detection. In contrast to the change seen in urinary NE, DOPA accumulation in brain tissues was unaffected by age or one hour of cold stress.

Uptake studies were carried out in vitro using diced (0.3 mm x 0.3 mm) hypothalamus, occipital cortex, and striatum. Tissues were suspended in Krebs buffer, which had been oxygenated with 95%/5% $O_2/CO_2$. They were incubated at 34-36.5°C under a constant flow of $O_2/CO_2$ in a shaking water bath. After a 10 minute preincubation with or without an uptake inhibitor ($10^{-5}$ M desipramine), $^3$H-norepinephrine was added to hypothalamic and cortical samples to give a final concentration of $10^{-7}$ M. $^3$H-Dopamine was added to striatal samples preincubated with or without $10^{-5}$ M nomifensine to give a final tracer concentration of $10^{-7}$ M. The samples were then incubated an additional 7 minutes. Uptake was stopped by placing the incubation tubes in ice water. As an additional control, samples were placed in the ice bath prior to the addition of tracer. (Supported by NS16215, DA02668 and NIA.)

280.4 STRIATAL AND MESOLIMBIC DOPAMINE ($D_2$) RECEPTORS IN RAT BRAIN AS DETERMINED BY AGE, TIME AND ENVIRONMENTAL LIGHTING CONDITIONS. O. Pulido*and G. Rajakumar. Departments of Neurosciences and Psychiatry, McMaster University, Hamilton, Ontario, Canada L8N 3Z5.

Geriatric patients show a higher incidence of side effects and responsiveness to neuroleptic drugs than young individuals (Smith et al.: Aging, Vol.17, S.J. Enna et al., Eds. Raven Press, N.Y., 1981, p.231; Borchinsky, S.: Exp. Gerontol. 19:227, 1984). The clinical potency of neuroleptics correlates closely with the affinity of binding for the $D_2$ dopamine receptors (Seeman et al., Nature 261:717, 1976). We have studied the population (Bmax) and affinity (Kd) of dopamine ($D_2$) receptors by $^3$H-spiroperidol-binding in striatum and mesolimbic region (olfactory tubercle and nucleus accumbens) of young (3-month-old) and old (20-month-old) male Long Evans rats. Animals were kept under a 14L:10D lighting cycle, for 3-8 wks prior to sacrifice. Animals were decapitated at 4 or 10 hrs after lights were on and 3 or 7 hrs after lights were off. Brains were rapidly removed, dissected and frozen at -70°C until used. Direct radioligand binding with $^3$H-spiroperidol was carried out on crude membrane preparation of striatal and mesolimbic brain regions from young and old animals, following the same procedure used by Chiu et al. (Europ. J. Pharmacol. 82:243, 1982). Concentrations of 0.05 - 0.8 nM were used in the presence and absence of 1 uM (+) butaclomol to define nonspecific binding. Results indicate that age, time and lighting conditions do not significantly affect or modify the Bmax or Kd of $D_2$ receptors in the mesolimbic area. However, Bmax ($P=0.0039$, $DF=4.9$) and Kd ($P=0.045$, $DF=2.1$) of $D_2$ receptors in striatum from old animals showed a sharp increase from 3 pm (light) to 10 pm (dark) followed by a fall at 2 am (dark). The lowest values were seen during the light phase. In young animals $D_2$ receptors showed similar rhythmic patterns, but the spike at 10 pm was not statistically significant from the values observed at other times.

Striatal $^3$H-spiroperidol Binding

| | Young | | Old | |
|---|---|---|---|---|
| | 3 pm | 10 pm | 3 pm | 10 pm |
| Kd (nM) | 0.387 | 0.502 | 0.424 | 3.11 |
| Bmax (fmol/mg protein) | 196 | 246 | 164 | 474 |

The spike at 10 pm of the striatal $D_2$ receptors Bmax ($P=0.0016$, $DF=4.4$) and Kd ($P=0.049$, $DF=2.3$) was significantly higher in old than young animals. These preliminary results show that aged animals exhibit more prominent circadian rhythms in Bmax and Kd of striatal $D_2$ receptors when compared to young animals, indicating that both age and time are important variables to be considered for drug response.

This work was supported by The Gerontology Research Council of Ontario.

**280.5** LOW LEVEL (SUBNORMAL) CHRONIC DIETARY CHOLINE SUSTAINS RETENTION OF LEARNING IN AGING MICE. Ronald F. Mervis. Division of Neuropathology, The Ohio State University College of Medicine, Columbus, Ohio 43210

The affect of chronic choline availability on behavior in aging C57Bl mice (/6J and /6NNIA strains) was evaluated using a passive-avoidance paradigm to assess 24 hour retention of learning. A diet containing very low levels of choline (0.9 mg/gm) was found to result in impaired retention of learning. However, an isocaloric and isonitrogenous diet containing 1.5 mg/gm of choline -- well below the levels in standard rodent chow preparations (2.3 mg/gm) -- was found to result in an improvement of retention of learning that was at least equal to or even surpassed the performance of mice on various choline enriched diets (2.4, 4.8 or 10.8 mg/gm of choline). Wecker and Trommer (J. Neurochem. 43:1762-1765, 1984) have proposed that transport of choline through the blood-brain barrier may be modulated by a choline stimulated inhibitory substance in serum. This suggests that the subnormal levels of dietary choline which are approximately equivalent to minimal dietary requirements may be too low to stimulate production of the inhibitory factor, and hence are transported rapidly into the brain. Higher levels of dietary choline, however, could result in the formation of the inhibitory factor which would lower transport of available choline to the brain. Therefore, higher levels of dietary choline did not produce comparable improvement in passive-avoidance retention.

**280.6** Behavioral Responsiveness to Estrogen in Female Rats: Influence of Strain and of Aging.
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There have been conflicting reports on the female rat regarding aging lordosis behavior and sensitivity to gonadal steroids. While investigating these relationships in Fischer (F) rats it became apparent that a substantial percentage (43%) of our aged CDF F-344 (Charles River) rats (20 mo.) were unusually sensitive to doses of Estradiol Benzoate (EB) previously used in this lab on another strain. We therefore decided to compare estrogen sensitivity in young (3-4 mo.)(N=17) and aged (21-22 mo.)(N=18) female F rats. To explore strain differences we also included young Sprague-Dawley (SD) rats from another supplier (Simonsen)(3-4 mo.)(N=13). All subjects were ovariectomized and placed in a reverse light room for 2 wks. Subjects were then randomly assigned to either a low dose EB (0.5ug x 3 days) or a higher dose EB (2.0ug x 3 days) and tested for lordosis behavior in a plexiglass arena under red light illumination with two sexually vigorous males on the 4th day. Behavior was scored by two independent investigators. A 2 wk. gonadal steroid free period followed, then the animals were re-tested in the same manner to note experiential effects. After testing, all subjects were weighed and sacrificed. Organs were inspected and weighed to evaluate general health status.

| | | Test One | | Test Two | |
|---|---|---|---|---|---|
| EB x 3 days | | 0.5ug | 2.0ug | 0.5ug | 2.0ug |
| S.D. | $\bar{X}$ | 2.50 | 17.86 | 5.83 | 19.00 |
| 3-4 mo. | ± | 2.28 | 4.61 | 3.80 | 6.66 |
| Fischer | $\bar{X}$ | 19.38 | 44.89 | 56.13 | 84.56 |
| 3-4 mo. | ± | 4.00 | 7.14 | 7.87 | 2.62 |
| Fischer | $\bar{X}$ | 33.13 | 49.20 | 70.83 | 76.60 |
| 21-22 mo. | ± | 10.28 | 11.07 | 6.69 | 8.48 |

Although F old subjects were more sensitive to EB (higher Lordosis Quotient, LQ) than young F, differences were not statistically significant. However, in all cases SD young were significantly less sensitive than both young and old F rats. Furthermore, both F young and old showed a significant increase in LQ for both dosages in the second test, while the SD did not.

This study suggests that aging does not decrease estrogen sensitivity in the F rat, but does point to a significant strain difference in estrogen sensitivity between F and SD rats. Furthermore, only F rats show significant increase in EB sensitivity with repeated testing at these doses, which may reflect their higher sensitivity.

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**280.7** CIRCADIAN RHYTHM OF LOCAL CEREBRAL GLUCOSE UTILIZATION (LCGU) IN ESTRADIOL-TREATED OVARIECTOMIZED RATS: EFFECTS OF AGE. P.M. Wise[1], E.D. London[2], I.R. Cohen-Becker[1], and N.G. Weiland[1]. [1]Dept. of Physiology, University of Maryland, School of Medicine, Baltimore, MD 21201 and [2]Neuropharmacology Lab., NIDA Addiction Research Center, Baltimore, MD 21224.

Previous reports have demonstrated the presence of a circadian rhythm in LCGU in the suprachiasmatic nucleus (SCN) of male rats (Schwartz, W. et al., J. Comp. Neurol., 189:157,1980). The ability of estradiol to induce LH surges depends upon an intact SCN and is altered during middle-age. To determine whether changes in the circadian pattern of neural function could account for age-related differences in the pattern of LH surges, we measured LCGU in several brain areas, with particular emphasis on hypothalamic areas involved in cyclic LH release.

Young (3-4 mo) and middle-aged (11-14 mo) cycling rats were ovariectomized. One week later (day 0), they were implanted with Silastic capsules containing estradiol, which produced physiological concentrations of estradiol in serum. On day 1, the external jugular vein was cannulated to the level of the right atrium, and the cannula was externalized through the nape of the neck. LCGU was measured by the autoradiographic 2-deoxy-D-[1-$^{14}$C] glucose method (Sokoloff, L. et al., J. Neurochem., 28:897,1977) in undisturbed, unrestrained rats at 0300, 0500, 1000, 1200, 1400, 1700, 1900 and 2300h (lights on between 0400-1800h). We observed a circadian LCGU pattern in the SCN of young rats: LCGU was higher during the light phase compared with the dark period. At 1000h there was a transient decline. This decline occurred at a time just prior to the previously reported initiation of LH surges and increases in norepinephrine activity (Wise, P.M., Endocrinol., 115:801,1984). In contrast, although middle-aged rats exhibited a circadian rhythm in SCN-LCGU, there was no decrement at 1000h, and LCGU decreased to a lower level at 1400 and 1700h as compared with young rats. Similar trends in circadian rhythmicity were observed in the medial preoptic nucleus, suprachiasmatic preoptic nucleus and paraventricular nucleus. LCGU in middle-aged rats was lower during the afternoon in these hypothalamic nuclei. Other brain areas which exhibited an age-related decline in LCGU were: cortical areas, corpus striatum, corpus callosum, nucleus accumbens, bed nucleus of the stria terminalis, amygdala, dorso- and ventromedial nucleus and lateral aspect of the medial mammillary nucleus.

These data indicate that there are changes in the circadian pattern of LCGU in the suprachiasmatic nucleus which relate to the timing and amplitude of estradiol-induced LH surges in middle-aged rats. The data suggest that a change in the biological clock occurs with age and that this change affects the ability of rats to exhibit cyclic reproductive function. (Supported in part by NIH AG-02224 and AG-00168 to PMW)

**280.8** PROGESTERONE PREVENTS AGE-ASSOCIATED LENGTHENING OF ESTROUS CYCLES AND ATTENUATION OF PREOVULATORY GONADOTROPIN SURGES IN FEMALE RATS. L.V. De Paolo* and K.L. Rowlands* (SPON: B. Brooks). Dept. of Physiol. Univ. of TX. Hlth. Sci. Ctr., San Antonio, TX 78284.

Recently, Nass et al. (Biol. Reprod. 27:609, 1982) reported that mating delayed the age-associated decline in reproductive function of female rats. Since circulating progesterone (P) levels are elevated for a 2-3 week interval during pregnancy, the following study was conducted to determine whether intermittent elevations in P levels can alter the rate of reproductive aging in female rats. Beginning at 2 mos of age, 4-day cycling rats were divided into two groups. In one group (n=22), each rat was inserted s.c. with 3 Silastic capsules containing crystalline P while rats in another group (n=45) received 1 empty capsule. After 2 weeks, the capsules were removed for 2 weeks. Thereafter, implantation and removal of capsules were repeated 5 additional times. Rats receiving P capsules became acyclic 3-4 days after exposure to P and resumed cyclicity 4-7 days after P-capsule removal. One month after removal of the last series of capsules (rats approximately 8-mos old), rats exhibiting consecutive 4-day cycles were bled at 4-h intervals from 1400h on proestrus (Pr) to 1000h on estrus (E). At 1600h E, rats were sacrificed and trunk blood was collected. Plasma levels of LH and FSH were determined by RIA. For comparison, a group of 3-mo-old rats (n=14) was bled on Pr and E. In 8-mo rats who received empty capsules, 26.7% of these rats exhibited 4-day cycles compared to 65.6% of 3-mo rats. However, in contrast to rats who received empty capsules, 63.1% of P-treated rats exhibited 4-day cycles. In comparison to 3-mo rats, preovulatory LH and FSH surges were attenuated in 8-mo rats given empty capsules. Furthermore, plasma FSH levels in these 8-mo rats were lower at 2200h Pr and during the secondary FSH surge at 0200 and 0600h E than levels observed at these times in 3-mo rats. However, by 1600h E, plasma FSH levels were higher in 8-mo rats not receiving P than in 3-mo rats. In striking contrast, preovulatory LH and FSH surges were indistinguishable between 3-mo rats and P-treated rats. Moreover, profiles of plasma FSH levels were similar on Pr and E in these two groups except at 1000 and 1600h E when plasma FSH levels were higher in P-treated rats. These results demonstrate that age-associated lengthening of estrous cycles and impairments in neuroendocrine mechanisms regulating preovulatory LH/FSH surges can be prevented at least temporarily by previous intermittent exposure to P and suggest that the disruption of estrous cycles and preovulatory surges in older rats are not related to chronological age per se. In contrast, the inability of P to prevent altered FSH secretion during the latter portion of the secondary FSH surge suggests that impairments in the mechanisms controlling this rise are attributed to aging per se. (Supported by NIH grant AG-03764).

280.9 IN LIFESPAN RESEARCH, ONE HOUR OF ENVIRONMENTAL ENRICHMENT PER MONTH HAD CUMULATIVE EFFECTS ON MARKING BEHAVIOR IN GERBILS AFTER TEN MONTHS OF AGE. MaryLou Cheal. Department of Psychology, Arizona State University, Tempe, AZ 85287.

In many experiments, housing conditions have been shown to influence behavior of experimental animals. Plasticity of the brain of young animals due to environmental factors has been clearly demonstrated. More recently, even the brains of old animals have been shown to be susceptible to change after environmental stimulation. In this research, the effects of repeated short exposures to environmental enrichment across the lifespan were studied. In a longitudinal study, 62 gerbils were reared in groups housed in identical cages with climbing apparatus and food and water ad libitum. Half of the gerbils were placed outdoors in a large desert environment for one hour each month from two months of age throughout the lifespan. No behavioral differences were found between gerbils given enrichment and gerbils in the indoor control condition during development and young adult life. However, starting at ten months of age, gerbils given the outdoor experience made more marks with the ventral gland than did controls. This species-specific behavior pattern is sexually dichotomous; i.e., male gerbils mark more than female gerbils. Nonetheless, the enrichment effect occurred in both male and female gerbils. The ventral glands of these gerbils developed at younger ages than those of controls (Soc. Neurosci. Abst., 1984, 10, 452), but there were no differences in the size of the ventral glands at the ages when marking behavior was enhanced. This is the first demonstration of cumulative effects of brief outdoor exposures on behavior. Because this behavior is known to be dependent on gonadal hormones, these data also suggest that neuroendocrine events can be modified by the environment in adult animals.

280.10 HIGHER-ORDER MOTOR FUNCTIONS IN ALZHEIMER'S DISEASE. S. Stockmeyer,* S. Corkin, & J. H. Growdon. MIT, Cambridge, MA 02139, Massachusetts General Hospital, Boston, MA 02114.

Primary motor disorders are not common in Alzheimer's disease (AD), but deficits in higher-order control functions have been described. Corkin, Growdon, & Sullivan, 1981, found that patients with AD were impaired on a coordinated tapping task but not on repetitious fine-finger motions. In order to extend this observation, we used two types of tasks that could reveal problems in planning or organizing motor acts.

Patients with AD ( N = 34 ) and healthy control subjects subjects ( N = 20 ) were examined for the presence and severity of mirror synkinesia in the hands during finger movements. The subject depressed keys attached to potentiometers that signaled any release of pressure. The keys were held depressed by the fingers of one hand while one finger of the opposite hand was either actively lifted or performed a tapping movement. As expected, control subjects showed a small amount of synkinesia; in contrast, patients with AD demonstrated significantly greater synkinesia in both hands ( $P < .05$ ). The presence of excessive mirror synkinesia indicates a lack of inhibition of the ipsilateral pyramidal tract.

In a modification of the Thurstone Tapping Test subjects were required to coordinate both hands temporally and spatially as they tapped in circular, vertical, and horizontal movements. Mirror and nonmirror movements in clockwise and counterclockwise directions were included in the task variations. The same movements were also performed unilterally. The ratios of unilateral to bilateral tapping on six task variations were significantly higher for patients with AD than for control subjects ( $P < .05$ ). Although most patients with AD could perform the task unilaterally, many were noticeably impaired in speed and coordination in the bilateral condition. Control subjects reduced their speed in the bilateral condition as expected, but were able to maintain the coordination required by the task. These observations indicate that patients with AD are unable to monitor the sensory cues and motor output for both hands simultaneously.

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280.11 HIPPOCAMPAL PYRAMIDAL CELLS AND AGING: A NEURONOMETRIC STUDY. D.V. Jeste, T. Bryant, C. Owen and J.B. Lohr. (SPON: R.J. Wyatt). Neuropsychiatry Branch, NIMH, Intramural Research Program, Saint Elizabeths Hospital, Washington, D.C. 20032.

**Introduction:** It is now generally believed that certain regions of the brain manifest a selective vulnerability to the effects of aging. Previous studies of age-related neuronal loss in hippocampus have yielded conflicting results. Whereas Ball (Acta Neuropath. 5:249, 1977) and Dam (J. Neuropath. Appl. Neurobiol. 5:249, 1979) found a loss of hippocampal pyramidal cells with aging, Devaney and Johnson (Gerontology 30:100, 1984) reported an apparent increase in the density of hippocampal neurons with age. The present study was undertaken to determine aging-associated changes in density and size of pyramidal cells in each of the four sectors (CA1 thru CA4) of right and left hippocampi.

**Material and Methods:** We examined brains of 20 subjects (11 females, 9 males, age range 6-94, mean 48 ± 29 years) from the "normative" series in the Yakovlev Collection, at the Armed Forces Institute of Pathology, Washington, D.C. We chose sections of right and left hippocampi according to specified atlas criteria. Using Zeiss Videoplan-II (a computerized planimeter), we measured areal density, neuronal cross-sectional area and nuclear area in sectors CA1 thru CA4. These measurements were done "blind" with respect to the clinical data.

**Results:** There were no significant differences in the parameters studied, between males and females or between right and left hippocampi. There was no significant correlation between age and neuronal density, cell cross sectional area or nuclear size in any of the 4 sectors. Subjects over 65 (n=6) had lower mean neuronal density than those under 65 (n=14), although this difference was significant only for CA4 (23.2 ± 5.5 vs 29.4 ± 5.7 cells/unit area; two-tailed $t = 2.101$; $p < 0.05$).

**Comment:** Our study does not support the reports of significant alterations in hippocampal pyramidal cells with aging, with a possible exception of neuronal density in CA4. This is in sharp contrast to our earlier work suggesting a significant decrease in the density of cerebellar Purkinje cells with age (Jeste, Lohr, Mani, et al., In "Current Perspectives on Dementias" ed. DV Jeste, American Psychiatric Press, Washington, D.C., in press). We will discuss limitations and possible implications of our results.

280.12 HIRANO BODY FILAMENTS CONTAIN ACTIN AND TROPOMYOSIN. P.G. Galloway*, G. Perry* and P. Gambetti. Division of Neuropathology, Institute of Pathology, CWRU, Cleveland, Ohio 44106.

Hirano bodies, rod-shaped eosinophilic inclusions found in the hippocampus and other parts of the nervous system, occur in normal aging but are increased in Alzheimer's disease and other neurological disorders. At the ultrastructural level, the Hirano body is composed of lamellar filaments similar in size to actin filaments. The presence of actin in Hirano bodies has been previously described at the light microscopic level. The present study was undertaken to determine the ultrastructural localization of actin and actin binding proteins in the Hirano body. Immunoperoxidase staining of paraffin-embedded hippocampal tissue with a poly- and a monoclonal antibody to chicken gizzard actin confirmed the presence of actin antigen in the Hirano body at the light microscope level. For ultrastructural analysis, free-floating vibratome sections were stained with both antibodies by using the peroxidase-antiperoxidase and indirect immunogold procedures. Sections were osmicated, embedded in Spurr's medium and thin sectioned. At the electron microscope level, immunostaining of Hirano body filaments was observed with the actin antibodies. Staining could be blocked by adsorbtion with a chicken gizzard actin preparation. The presence of actin binding proteins in the Hirano body was investigated using antibodies to tropomyosin, spectrin and myosin. The antiserum to tropomyosin stained the Hirano body. Further investigation as to the arrangement of actin and tropomyosin antigen sites within the Hirano body will clarify the participation of these proteins in the formation of this cytoplasmic inclusion.

280.13 MULTIPLE TRANSMITTER-SPECIFIC MARKERS IN SENILE PLAQUES IN ALZHEIMER'S DISEASE. R. G. Struble, R. E. Powers*, M. F. Casanova*, C. A. Kitt, D. T. O'Connor*† and D. L. Price. Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Baltimore, MD 21205; †Department of Medicine, University of California, San Diego School of Medicine, San Diego, CA 92093.

Senile plaques, one of the major histopathological hallmarks of Alzheimer's disease (AD), are composed of neurites and amyloid. Neurites are enlarged axons, terminals, and dendrites, and some have been shown to be derived from cholinergic-, somatostatinergic-, or substance P-containing neurons. However, the transmitter specificity of neurites in specific regions of brain have not been systematically examined for multiple neurotransmitter markers.

In the present immunohistochemical study, we examined the character and distribution of fibers stained for dopamine ß-hydroxylase, somatostatin-14, substance P, cholecystokinin, neurotensin, vasoactive intestinal peptide, bombesin, and leucine-enkephalin in the hippocampi and adjacent cortical regions of five patients with clinical and pathological diagnoses of AD and five controls, ranging in age from 35-78 years. In controls, we plotted the normal distribution of stained cells/fibers. To date, in the AD cases, we have observed neurites showing all of the above transmitter-specific markers. Some neurites were in proximity to amyloid, suggesting that these neurites were associated with senile plaques. Hence, fiber abnormalities and neurites in senile plaques were seen with antibodies directed against a variety of transmitter markers. The distribution of stained neurites was roughly comparable to the density of fibers in the particular transmitter system in normal tissues, i.e., where fibers of a particular system were normally abundant, there appeared to be a greater possibility that the system participated in the formation of plaques. Adjacent silver-stained sections disclosed many more plaques than seen by immunocytochemical methods, suggesting that neurites of a variety of other transmitter systems may show similar structural pathology. Mechanisms leading to the formation of plaques are not known but could represent a local response to loss of specific transmitter systems arising from subcortical regions (e.g., basal forebrain or brainstem) or reflect local cortical abnormalities. These observations, that neurites expressing epitopes of transmitter systems known to be at risk in AD (e.g., somatostatin or substance P) and systems not reported to be decreased (e.g., cholecystokinin) in AD, suggest that neurites in plaques may represent both degenerating systems and possibly abortive attempts at regeneration by local neuronal processes.

## ACTION POTENTIALS AND ION CHANNELS VII

281.1 DIVALENT CATION EFFECTS ON NMDA-ACTIVATED CHANNELS CAN BE DESCRIBED AS Mg-LIKE OR Ca-LIKE. L.M. Nowak and P. Ascher*, Dept. Pharmacology, Cornell U.,Ithaca, NY and *Lab. de Neurobiologie, Ecole Normale Supérieure, Paris 75005.

N-methyl-D-aspartate (NMDA) activates a class of glutamate receptors in vertebrate CNS and increases a cationic conductance. In physiological solutions the current-voltage (I-V) relationship has a distinct non-linearity, such that responses at -60mV are smaller than those at -40mV despite the reversal potential being at 0mV. Previously we demonstrated that this region of negative-slope conductance in the I-V curve actually was due to a voltage-dependent blockade of the channels by extracellular $Mg^{++}$ions ($[Mg^{++}]_o$ ≤1mM)(Nowak et al.1984, Nature 307:462). We have extended these studies, examining the actions of other divalent cations on NMDA-activated single channels.

Outside-out membrane patches were excised from somata of mouse brain neurones grown in primary dissociated cell culture. Patch pipettes contained (in mM): 140 CsCl, 5 EGTA-K, 0.5 $CaCl_2$, 10 Hepes-K (pH7.2). 10mM EGTA and 1mM $CaCl_2$ were used in some experiments. The outside surface was superfused with a solution containing (in mM): 140 NaCl, 2.8 KCl and 10 Hepes-Na (pH7.2) to which was added 10µM NMDA to stimulate channel openings and various amounts of divalent cations.

Without divalent cations NMDA-activated channels had a mean open time near 5ms (20-24°) and a linear conductance of 50pS. Although all divalent cations tested reduced the currents measured at negative potentials the results obtained were clearly separated into two groups of effects which could be termed Mg-like and Ca-like. As previously reported, $[Mg^{++}]_o$ alters the kinetics of NMDA-activated channels. Addition of 10-100µM $[Mg^{++}]_o$ resulted in channel openings which were interrupted by brief, fast, closures giving the impression of bursts. Rapid closures were longer with hyperpolarization. The burst duration and the number of bursts decreased with increasing $[Mg^{++}]_o$ and with increasing hyperpolarization. For low $[Mg^{++}]_o$ channel conductance was unchanged. In contrast $Ca^{++}$ was only effective at high concentrations (5-10mM) where its major action was to decrease single channel conductance. Channel kinetics were unchanged between -60 and +60mV. The decrease in channel conductance was slight at positive potentials, pronounced between 0 and -60mV, and moderate below -60mV. The $Mg^{++}$ effects were mimicked by $Co^{++}$, $Mn^{++}$, and $Ni^{++}$at similar concentrations (10-100µ M) while 10mM $Ba^{++}$ exhibited the Ca-like effect.

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281.2 A NEW PATCH-CLAMP CONFIGURATION: SLOW WHOLE CELL. M. Lindau*, J.M. Fernandez* and E. Neher*. (Spon. W. Almers). Abt. Membranbiophysik, Max-Planck-Institut für biophysikalische Chemie, D-3400 Göttingen, W.-Germany.

Cells, internally dialyzed with patch pipette solutions very often lose some of their functional properties. It is suspected that this is due to the loss of regulatory proteins or other cellular components that are essential to the regulation of cell function by second (intracellular) messengers. For example, intact rat peritoneal mast cells respond to antigens or substances like compound 48/80 by degranulating violently, releasing histamine and other mediators. But dialyzed mast cells invariably failed to degranulate in response to external stimuli, when, in the whole cell mode, the access resistance was 1 - 20 MΩ.

If a cell-attached patch was formed with pipette solutions containing Ca buffers, ATP and no added Ca, the patch very often became leaky within several minutes of recording. A voltage pulse applied to the pipette then induced a current transient similar to the capacitive transient obtained in fast whole cell, but about 100 times slower and smaller. As in the whole cell configuration the capacitance and conductance of the cell could be measured from this transient, revealing normal values for capacitance of 5 - 10 pF and a cell input resistance typically between 20 - 100 GΩ; the pipette series resistance ranged between 100 - 5000 MΩ which is still more than an order of magnitude smaller than the cell input resistance. We call this configuration "slow whole cell" as opposed to the usual "fast whole cell". We believe this configuration is obtained when the plasma membrane (normally 100 kΩ·cm²) is damaged upon patch formation, however is not entirely disrupted thus keeping the intracellular environment unchanged.

Mast cells monitored in the slow whole cell mode, degranulated in response to antigens and 48/80, showing a 3-fold increase in the cell capacitance and frequently showing no increase in the specific membrane conductance. The latter result questions the view that the opening of Ca-channels mediate antigen-induced histamine release from mast cells.

Preliminary experiments indicate that the slow whole-cell configuration can be reached in a variety of cell types by including in the pipette solution α-toxin from staphylococcus aureus which is known to produce well defined holes in membranes.

We thank Prof. S. Bhakdi, University of Giessen, F.R.G., for a sample of purified α-toxin.

281.3 IONIC INTERACTIONS WITHIN THE VESTIBULES OF ION CHANNELS

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Many channels have wide, funnel-shaped vestibules that lead to a more selective, narrow region. The AChR pore, for example, has a large vestibule and is thought to be lined by hydrophilic amino acids which include tens of negatively and positively charged residues, but has only a small net charge. Due to the size and net charge it is likely that the vestibule contains many ions, and in the widest areas the molecular behavior is much like bulk solution. As the vestibule funnel narrows, ions experience a driving force due to the potential difference across the membrane, and near the narrow region ion-ion and ion-channel interactions become more intense. These considerations suggest a progressive shift from a bulk-like to a selectivity-filter situation, with the funnel-shaped vestibule serving as the transition zone. Most effort has been directed at describing interaction within the narrow region, because it is the place where the ion must closely approach the pore walls. Here, however, attention is focused on the vestibule, which is considered to have net charge, a funnel shape, and to be in equilibrium with the bulk solution. Ion size, ionic screening of the net charge, binding, and blockage of current are considered in determining how the vestibule provides a "conditioned" environment for the narrow, selective region.

Depending on the experimental conditions, the vestibule gives varied effects: 1. One benefit of a wide vestibule is that the narrow, restrictive region can be very short, permitting the channel's maximum conductance (Gmax) to be larger. 2. Net charge inside the vestibule creates a local potential that confers some cation vs. anion and divalent vs. monovalent selectivity. 3. Large organic ions cannot fit into the vestibule at as high a concentration as small ions, so they are less effective at screening the potential due to the vestibule's charge. Therefore, at an equivalent bulk concentration of large ions the vestibule's attractive potential is larger, causing these ions to saturate sites inside the channel at a lower bulk concentration. This factor may contribute to the relatively high affinity block of current by large impermeant cations such as TEA+ or some local anesthetics. 4. The potential produced by a net charge in the vestibule varies as a function of ionic concentration. This produces conductances at low concentrations that are larger than expected from measurements at high concentrations, an effect that also can be produced by multiple-ion occupancy of the narrow region. 5. The apparent Gmax and Kd of a permeant ion can change due to interactions in the vestibule caused by adding mM concentrations of divalent or large ions to the bulk solution.

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281.4 TWO PHARMACOLOGICALLY DISTINCT $Ca^{++}$-ACTIVATED $K^+$ CURRENTS ARE PRESENT IN THE GH3 ANTERIOR PITUITARY CELL LINE.

A.K. Ritchie* (SPON: Lawrence W. Haynes). Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77550.

The single electrode, patch voltage clamp technique was used to study whole cell currents in the GH3 anterior pituitary cell line. GH3 cells have an outward current that is activated by the voltage dependent entry of $Ca^{++}$ ions into the cell. This outward current is absent in $Ca^{++}$-free, EGTA saline and is inhibited by $Ca^{++}$ channel blockers such as 0.5 mM $Cd^{++}$ (Dubinsky, J.M. and G.S. Oxford, J. Gen. Physiol. 83:309-339, 1984). Tail current analysis reveals that this $Ca^{++}$-activated outward current has a reversal potential of -72 ± 2 mV ($\bar{x}$ ± SEM, N = 7) in standard saline (5.6 mM KCl, 150 mM NaCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM glucose, 10 mM HEPES, 5 mM 4-aminopyridine, 3 μM TTX, pH 7.3) when using an intracellular electrode that contains 150 mM KCl and 3 mM HEPES, pH 7.3. At room temperature the tail current reversal potential changes by 56 mV/decade change in external $K^+$ concentration thus confirming that the $Ca^{++}$-activated outward current is mediated by a selective increase in $K^+$ ion permeability. The majority of the $Ca^{++}$-activated $K^+$ current that is elicited by a 400 mS step to +20 mV is inhibited by extracellular TEA with a half maximal inhibitory concentration of 0.8 mM. However, in the presence of a maximally effective concentration of TEA (30 mM), a small portion (10-20%) of the $Cd^{++}$ inhibitable outward current persists. The following evidence indicates that this $Cd^{++}$ inhibitable, but TEA resistant, outward current is also a $Ca^{++}$-activated $K^+$ current: 1) the tail current amplitude measured upon returned to the holding potential of -50 mV and plotted as a function of the voltage used to elicit this outward current has a voltage dependence that is similar to that of the inward $Ca^{++}$ current in GH3 cells and 2) the reversal potential of the tail current for this outward current is -72 mV.

The TEA resistant $Ca^{++}$-activated $K^+$ current is inhibited by 100 nM apamin. Apamin is a polypeptide component of bee venom which has been shown by others to be an inhibitor of a relatively TEA resistant component of the $Ca^{++}$-activated $K^+$ conductance in rat myotubes (Romey, G. and M. Lazdunski. Biochem. Biophys. Res. Comm. 118: 669-674, 1984) and bullfrog sympathetic ganglion cells (Pennefather, P., B. Lancaster and P.R. Adams. Soc. Neurosci. Abs. 10: 147, 1984). In the absence of TEA, apamin inhibits 10 to 20% of the total $Cd^{++}$ sensitive outward current that is elicited by a voltage step to +20 mV. Both the TEA and apamin sensitive $Ca^{++}$-activated $K^+$ currents can also be activated at -50 mV in a $Ca^{++}$-free, EGTA saline by application of 50 nM thyrotropin releasing hormone, a tripeptide which has been shown in GH3 cells to cause the release of $Ca^{++}$ from intracellular stores.

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281.5 PATCH CLAMP ANALYSIS OF INACTIVATING OUTWARD CURRENTS IN DISSOCIATED CNS NEURONS OF WILD-TYPE AND *SHAKER*$^5$ *DROSOPHILA*.

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Patch clamp analysis of ion channel mutants in *Drosophila* affords a powerful approach to the correlation of structure and function in membrane channel proteins. Previous voltage clamp studies of the dorsal longitudinal flight muscles (DLM) have shown that the $Sh^5$ locus mutation alters the kinetic properties of the fast transient potassium current $I_A$ (Salkoff and Wyman, TINS. 6:128, 1983). We report here experiments using the whole-cell and cell-attached patch clamp techniques to record inactivating outward currents in dissociated CNS neurons from wild-type and $Sh^5$ mutant flies.

Primary CNS cultures were prepared from late third instar larvae as described previously (Wu et al., J. Neurosci. 3:1888, 1983). Experiments were performed on type II and type III neurons from one to eight day old cultures in a *Drosophila* Ringer supplemented with 10% Schneider's medium. Pipettes were filled with the same solution for cell attached experiments, or with a high $K^+$ low $Ca^{++}$ internal solution for whole-cell experiments.

Whole cell outward currents were recorded from both wild type and $Sh^5$ neurons. Cells from cultures less than two days old tended to have only the delayed current while cells from older cultures usually exhibited robust fast transient currents that were sometimes accompanied by delayed currents.

The fast transient outward current activated at -35 to -45 mV with a rapid, sigmoid and sharply voltage dependent time course, reaching its peak within 1.5 to 6 ms and then inactivating. The midpoint of the steady state inactivation curve was between -75 and -80 mV. The voltage dependence of activation was similar to values reported in the DLM, but the steady state inactivation curve was shifted in the hyperpolarizing direction by about 35 mV. In cell-attached patches, we have recorded ensembles of small, flickery single-channel events whose averages had kinetics consistent with the fast outward current.

The delayed outward currents appeared to be made up of more than one channel type. Prepulses to potentials depolarized from rest inactivated a component of the delayed current, but long test pulses failed to produce a falling phase in the current. Single channels with first latencies consistent with the characteristics of the inactivating component of the delayed current were observed in cell attached patches.

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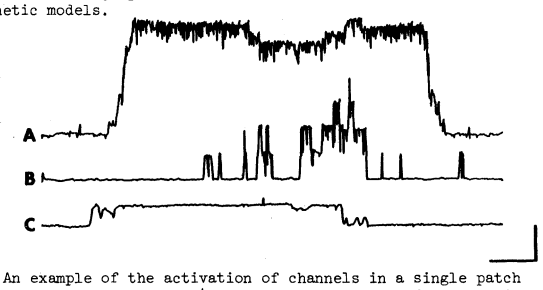
281.6 ACTIVATION OF POTASSIUM CHANNELS BY RAPID APPLICATION OF CALCIUM TO INSIDE-OUT PATCHES OF CULTURED HIPPOCAMPAL NEURONS.

R.S. Brett and B. Lancaster. Depts. of Anesthesiology and Neurobiology & Behavior, SUNY at Stony Brook, New York 11794

Ca-activated K channels mediate spike repolarization and after-hyperpolarization in a variety of excitable cells. To investigate the activation of K current by Ca at the single channel level, we are applying a method for the rapid (<10 ms) exchange of solutions bathing the cytoplasmic face of inside-out patches of cultured hippocampal neurons (cf. Brett, R.S., Dilger, J.P. & Adams, P.R., Neurosci. abstr. #71.1, 1984). Applications of 1-100 μM Ca for 200-1000 ms caused the appearance of 1-16 large-conductance (140-200 pS) channels. The latency and number of openings were dependent upon the Ca concentration (see figure). The probability of opening was also increased by depolarization. In symmetrical K solutions, the current reversed at zero mV. At negative pipette potentials, application of $BaCl_2$ (1 mM) to the cytoplasmic face rapidly and reversibly abolished Ca-activated channel openings. In occasional patches we have observed, together with events of the type shown, a second Ca-activated channel having lower conductance and different kinetics. The ability to make rapid Ca concentration jumps should allow new statistical tests of detailed kinetic models.




An example of the activation of channels in a single patch (pipette potential -50 mV) by rapid application of 1 and 100 μM Ca. Trace A, 100 μM Ca; calibration bar = 100 pA; Trace B, 1 μM Ca; calibration bar = 25 pA. Trace C, representation of the solution exchange profile after rupture of the patch, using a pulse of 70 mM KCl solution to alter the pipette tip potential. The horizontal bar = 100 ms.

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281.7 A VOLTAGE-DEPENDENT, 4-AMINOPYRIDINE SENSITIVE, OUTWARD CURRENT STUDIED IN VIVO IN CORTICAL NEURONS OF AWAKE CATS BY VOLTAGE SQUEEZE TECHNIQUES. C.D. Woody, V. Nenov*, E. Gruen*, P.Donley*, M.Vivian*, and W. Holmes. Depts. of Anatomy and Psychiatry, MRRC and BRI, UCLA Med. Ctr., Los Angeles, CA 90024.

Studies of 47 neurons of the precruciate cortex disclosed fast, outward currents that increased with increasing, positive step voltage commands from holding potentials set between -60 and -80 mV. Preceding depolarizing pulses reduced the currents while preceding hyperpolarizing pulses potentiated them. In 12 cells given pressure injection of 4-aminopyridine, all but one showed reduction of the outward current. The degree of reduction varied from cell to cell.

Electrodes used in these experiments were prepared from theta-capillaries and were tested for non-rectifying passage of + and - currents of >>20 nA prior to use. They were filled either with 3M KCl or with 1.5 - 2M 4-aminopyridine in 2.25M KAcetate. A Dagan 8100 amplifier was used for single electrode pulse clamping.

The measurements of changes in current corresponded with previous in vitro measurements in other cortical cells (Gustafsson, B., et al., Nature 299:252, 1982), and in Tritonia (cf. Thompson, S.H., J. Physiol. (Lond.) 265:465, 1977). The cable properties of neocortical pyramidal cells are such that an actual voltage clamp is unrealizable with control of but 1-10% of a space constant estimated from previous modeling of theoretical cable properties in cells of known morphology. Also, the fast sodium currents are incompletely controlled. Further, the large number of active synaptic conductances along the cable length results in a kind of counter clamping toward the normal resting potential of the cell. Hence, the potentials are squeezed away from the normal resting potential and towards the desired holding and command potentials.

Nonetheless, the preparation affords qualitative examination of currents and time courses found in the actual in vivo state and semi-quantitative measurements of sufficient precision and sensitivity to detect changes in outward currents upon administration of pharmacologic blocking agents.

Changes in outward currents have been linked to membrane changes supporting conditioning in invertebrates and potassium currents have been linked indirectly to ACh - cGMP dependent changes in membrane properties of the cells of this cortical region (which has been found necessary for some forms of mammalian conditioning). Thus, the present technical advance affords a possible means for detecting changes in currents related to mammalian conditioning. (Supported by AFOSR and NICHHD. We thank Dr. D. Alkon for invaluable help in beginning these studies.)

281.8 A SODIUM-ACTIVATED POTASSIUM CURRENT IN CULTURED VERTEBRATE NEURONES. D. Bertrand*, C.R. Bader and L. Bernheim* Dept of Physiology, University Medical Center, 1 rue Michel-Servet, CH-1211 Geneva 4, Switzerland.

Cultured neurones from dissociated avian parasympathetic and sensory ganglia were studied in voltage clamp with the whole cell recording technique. An outward current carried by potassium ions and suppressible by extracellular 4AP and TEA was found to be initiated whenever a sodium current entered the cell. This potassium current had the following characteristics : 1) it was suppressed by TTX at a concentration which blocked the fast inward sodium current, 2) it was suppressed by removal of extracellular sodium, 3) it could not be detected at voltages more depolarized than the sodium reversal potential, 4) inhibition of inactivation of the sodium current increased the duration of this potassium current, 5) it was strongly activated when the intracellular fluid contained 50 mM NaCl. These observations were made in the absence of extracellular calcium and in the presence of 5 mM EGTA and 5 mM ATP in the intracellular fluid.

We conclude that this potassium current is activated by an increase in the intracellular sodium concentration. Our results suggest that the quantity of sodium entering a cell during a single action potential is sufficient to activate this potassium current.

281.9 SINGLE TRANSIENT POTASSIUM CURRENTS IN MAMMALIAN SENSORY NEURONS STUDIED USING PATCH-CLAMP TECHNIQUES. H. KASAI* (SPON: K. TANAKA). Dept. of Physiol., Faculty of Medicine, Univ. of Tokyo, Tokyo 113, Japan.

A single-channel which carried transient outward current (A-current) was isolated in an extracellular patch-clamp electrode. Dorsal root ganglion cells isolated from adult guinea pigs were grown on poly-L-lysine coated plastic dishes for up to two weeks. Nerve cells were bathed in an isotonic KCl solution during the patch-clamp experiments (150mM KCl, 1mM $MgCl_2$, 1mM EGTA, 5mM Na-Hepes, 5.5mM glucose at pH7.4, 20-24°C), so that the resting membrane potential was close to 0mV. The patch pipette contained a Na-free Tris-solution (150mM Tris-HCl, 5mM KCl, 1mM $CaCl_2$, 1mM $MgCl_2$, at pH7.4). We chose those membrane patches from which A-current was purely recorded; when membrane potential was depolarized to 0mV for 30sec., A-current was completely inactivated, and thus, contamination of the patch with other channel currents was easily discriminated. The single-channel currents was elicited shortly after the membrane depolarization from hyperpolarized potential (-100mV). The appearance of the currents tended to reduce in time. The mean conductance of the K channel was 20.1±3.7 pS (mean±SD, n=15). The major charge carrier through the channel was determined as K ions. Mean open time was 6.8ms at 0mV. A closed time histogram was approximately fitted by two exponential curves with time constants 1ms and 3.4ms. A latency histogram for the first openings of the channel showed a peak at 2.5ms. These observations indicated the presence of two closed states in the process of activation of the channel. The activation and inactivation kinetics of this channel was studied at various membrane potential using ensemble averaged currents. The activation of the channel was started at potential>-60mV. The channel was inactivated by depolarization with two time constants, 100msec and 4sec. The complete inactivation was attained at potential>-40mV with slow time constant (4sec). Properties of the channel were not affected when mebrane patch was exposed to the Ca-free solution containing 1mM EGTA (inside-out patch). Effects of 4-aminopyridine (4-AP) were studied using the inside-out patch configuration; 1) 4-AP reduced the single-channel conductance (a half-reduction was attained at 12mM); 2) the reduction in the single-channel conductance was dependent on the membrane potential: it was larger at depolarized membrane potential; 3) 4-AP reduced the opening probabilty (a half-reduction was attained at 12mM at 0mV); 4) 4-AP shifted voltage-dependence of both the activation and inactivation of the channel in a depolarizing direction; 5) all of the effects were reversible.

281.10 OUTWARD CURRENTS INDUCED IN XENOPUS OOCYTES FOLLOWING INJECTION OF RNA FROM APLYSIA AND RAT BRAIN. M.B. Boyle, E. Azhderian* and L.K. Kaczmarek. Department of Pharmacology, Yale Univ. Sch. Med., New Haven, CT 06510.

It has been shown that when RNA isolated from vertebrate tissues is injected into oocytes of Xenopus laevis, the translation of the foreign mRNA may lead to the appearance of novel ion channels. We have examined the ability of the oocyte translation system to express ion channels after injection of RNA from the nervous system of the mollusc Aplysia. We have tested the electrical properties of Xenopus oocytes after injection of 1) poly(A+)-enriched RNA from rat brain; 2) total RNA from pooled cerebral, pedal, and pleural ganglia of Aplysia; and 3) total RNA from the peptidergic bag cell neurons of Aplysia. Total RNA was obtained by homogenization of the tissue in guanidine thiocyanate and centrifugation through a 5M CsCl cushion. The yield of total RNA per gram of starting material was 50 to 100 ug for Aplysia central ganglia. Oocytes were treated with 2 mg/ml collagenase at room temperature for 1 to 1.5 hours soon after isolation. Oocytes were later injected with 20 to 100 ng of RNA in water or 20 to 100 nl of sterile water. Ionic currents were measured 3 to 7 days after injection using 500 ms depolarizing steps from -40 or -100 mV. In oocytes injected with poly(A+)-enriched RNA isolated from rat brain, transient outward currents reaching peak in about 100 to 200 ms were observed, in agreement with the report of Gundersen et al. (Proc. Roy. Soc. Lond. B 220:131, 1983). In oocytes injected with RNA from pooled cerebral, pleural and pedal ganglia from the CNS of Aplysia, we observed delayed outward currents which activated during the first 50 to 200 ms and which showed no apparent inactivation with time. We have never observed similar currents in non-injected or water-injected controls. The injection of total RNA isolated from the peptidergic bag cells of Aplysia resulted in the appearance of a new transient outward current in 16 out of 26 oocytes from 3 frogs. This current was seen when the cells were held at either -40 or -100 mV. This current shows a faster activation than the transient current found in oocytes injected with RNA from rat brain. The amplitude of the current showed a monotonic increase with depolarization up to +60 mV. The current was not present in non-injected cells or in 8 water-injected controls from these frogs. However, in one animal not used in these experiments, a similar current was seen in non-injected cells. Thus, it remains to be determined whether these currents represent novel ion channels coded for by the injected RNA from Aplysia or whether the injections promote expression of native oocyte channels.

281.11 TWO TYPES OF DELAYED POTASSIUM CHANNELS: INACTIVATING AND NON-INACTIVATING CHANNELS. J. L. Ram & D. Dagan. Dept. of Physiology, Wayne State Univ., Detroit, MI 48201 & Rappaport Fam. Inst., Technion, Haifa, Israel.

Under voltage clamp of long duration, neurons show partial inactivation of voltage dependent delayed potassium current (IK). Channels and currents underlying IK were analysed in cell-attached patches of Helix neuronal cell bodies. Results indicate at least two principal types of IK channels, one which inactivates almost completely (IK,ID: inactivating delayed channels), and a second which does not show time-dependent inactivation (IK,ND: non-inactivating delayed channels). Fast outward transient (IA) channels have also been studied.

ID channels: Delay from onset of depolarization until peak number of open channels was >70 ms (mean=115 ms). Inactivation time constants averaged 350 ms. Opening probability decreased to near zero for long (1 s) depolarizations. Activation was voltage dependent and required >45 mV above rest. Single channel conductance was 45±12 pS. There are at least 2 closed states. With single channel patches, about half the responses were "blanks," consistent with a model in which channels can enter the inactive state directly without a previous opening. ID channels are not likely to be Ca-dep. K channels (IC), since ID channels are seen with 0 Ca ringer in the patch pipet. In other studies, Helix IC channels consistently have conductances of 15-20 pS, less than half the conductance of ID channels.

ND channels: Previous studies in Helix using fluctuation analysis (Reuter & Stevens, 1980) indicate non-inactivating channels of 2.5 pS, which would be too small to be seen as distinct channel openings and closings in our single channel records; however, one would expect a voltage dependent increase in patch mean conductance and an increase in current fluctuations with depolarization. This is exactly what is seen. This voltage dependent current begins to activate at about 50 mV above rest and does not inactivate over the course of 1 s. Steady state current is reached within 50 ms.

Thus, we propose that IK is due to at least two types of channels. Essentially all of the inactivating fraction of the current is due to ID channels; whereas, most of the non-inactivating current is due to ND channels.

IA channels: These have a low probability of activation from holding potential at rest but can be activated when holding potential is hyperpolarized 25 and 50 mV below rest. Peak opening probability at 75 mV above rest (from a holding potential 25 mV below rest) is reached in <15 ms and the inactivation time constant is <100 ms. Single channel conductance is 10-15 pS.

We thank the Lady Davis Trust for financial assistance to JLR.

281.12 $Ca^{2+}$-MEDIATED REDUCTION OF $K^+$ CURRENTS IS ENHANCED BY INJECTION OF $IP_3$ OR NEURONAL $Ca^{2+}$/CALMODULIN KINASE TYPE II. M.Sakakibara*, D.L.Alkon, J.T.Neary, R.DeLorenzo†, R.Gould¶, and E.Heldman§. Lab. Biophysics, NINCDS-NIH, MBL, Woods Hole, MA 02543; †Dept. Neurol., Yale Univ. Sch. Med., New Haven, CT 06510; ¶NY State Inst. Basic Res., Staten Island, NY 10314; §Dept. Pharmacol., Israel Inst. Biol. Sci., Ness-Ziona, Israel.

Elevation of intracellular $Ca^{2+}$, $[Ca^{2+}]c$, in the isolated soma of the Hermissenda type B cell has been shown to cause reduction of the early voltage-dependent outward current, $I_A$ (Alkon et al., Biophys. J. 40:245, 1982) and the $Ca^{2+}$-dependent outward current $I_C$ (Alkon and Sakakibara, Biophys. J., in press). The prolonged duration of $I_A$ and $I_C$ reduction (1-5 min) suggested that biochemical steps may be responsible for reduction which may outlast $[Ca^{2+}]c$ elevation itself and could lead to the $I_A$ and $I_C$ reduction (lasting days) which encodes associative memory. Previous studies showed that iontophoretic injection of one type of a $Ca^{2+}$/calmodulin (CaM) dependent protein kinase (PK), phosphorylase kinase, enhances and prolongs $Ca^{2+}$-mediated $I_A$ and $I_C$ reduction (Acosta-Urquidi et al., Science 224:1254, 1984). In the present study iontophoretic injection of neuronal $Ca^{2+}$/CaM-PK type II under isopotential conditions was found to cause significant $I_A$ (n=8, $p<.01$) and $I_C$ (n=8, $p<.02$) reduction lasting 20 min or longer. This reduction did not occur unless preceded by voltage-clamp conditions which caused significant $[Ca^{2+}]c$ elevation (25 sec depolarization paired with a 2 sec light of $\sim 10^4$ ergs/cm$^2$·sec). No such reduction occurred when the $Ca^{2+}$/CaM-PK was first inactivated before injection. $Ca^{2+}$/CaM-PK injection followed by $Ca^{2+}$ loads did not significantly alter $I_{Ca^{2+}}$ measured at 0 mV (in 0-$Na^+$, 300 mM $K^+$-ASW) the $K^+$ equilibrium potential, or the light-induced inward $Na^+$ current, $I_{Na^+}$. Trifluoperazine (10 μM) in the ASW caused increase of both $I_A$ and $I_C$ and shortening of $Ca^{2+}$-mediated reduction of outward $K^+$ current, measured as an apparently inward current (just opposite to the effects of $Ca^{2+}$/CaM-PK). Iontophoretic injection of inositol trisphosphate ($IP_3$) but not $IP_1$ produced $I_A$ and $I_C$ reduction even in the absence of $Ca^{2+}$ load conditions. $IP_3$-induced reduction of $I_A$ and $I_C$ also persisted for the recording duration. $IP_3$ injection caused a similar reduction of $I_A$ (n=8, $p<.005$) and $I_C$ (n=9, $p<.01$) measured across the isolated soma of the type A photoreceptor, but had no effect on $I_{Ca^{2+}}$ of either type B or A somata. $IP_3$ injection had no effect on $I_{Na^+}$ of type B cell but caused a clear increase of the type A $I_{Na^+}$ ($p<.001$). $IP_3$ injection, by mobilizing $[Ca^{2+}]c$, may produce its effects on $I_A$ and $I_C$ via activation of endogenous $Ca^{2+}$/CaM-PK and possibly endogenous C-kinase as well. The differences in $IP_3$ effects on type B and A $I_{Na^+}$ may reflect differences in endogenous enzyme levels which, in turn, might explain differences in receptor sensitivity during light and dark adaptation.

## DEVELOPMENT OF INVERTEBRATES II

282.1 TISSUE INTERACTIONS DURING MIGRATION OF NEURAL PRECURSORS IN LEECH EMBRYOS. S.A. Torrence* (SPON: D.K. Stuart) Dept. of Molecular Biology, U. of CA., Berkeley, Berkeley CA. 94720

In glossiphoniid leech embryos, neuroblasts and glioblasts that arise outside the prospective CNS migrate in small clusters along stereotyped pathways to reach their characteristic positions in and near the ventral ganglia. To examine the role of intercellular interactions in guiding migrating cells to their destinations and controlling their final positions, tissues offering potential guidance cues have been identified and ablated.

In each right or left hemisegment two groups of neural precursors belonging to the Q cell line migrate from the lateral edge of the body-wall rudiment toward the ventral midline where they produce the glia of the interganglionic connective, an antero-ventral cluster of central neurons, a single ventro-lateral central interneuron and a small cluster of peripheral neurons along one of the segmental nerve roots.

Among cells along the routes of migration that might provide guidance or positional cues are cells derived from two ectodermal cell lines (O and P) and from the mesoderm: several peripheral neurons, the nephridiopore, epidermal specializations called cell florets, and circular and longitudinal muscle fibers. In addition, cells of the N cell line, which gives rise to the majority of central neurons, have been proposed to play an important role in organizing the ganglia by Blair & Weisblat (1982, Dev. Biol. 91:64-72).

To learn which tissues are necessary for normal migration and positioning of neural precursors of the Q cell line, progenitor cells of various parts of the ectoderm or mesoderm have been ablated before the onset of migration, either by intracellular injection of the lethal enzyme DNase or by laser irradiation of cells labeled with a photosensitizing lineage tracer dye. Ectodermal ablations did not prevent migration and produced only subtle alterations in final cellular positions. Such alterations are probably a simple consequence of the overall reduction in ganglionic size. By contrast, partial ablations of the mesoderm produced substantial abnormalities in the positioning of cells after migration or prevented migration altogether, depending on the severity of the lesion. Thus normal migration and positioning of neural precursors from the Q cell line do not require specific interactions with other ectodermal cell lines but do depend on interactions with the mesoderm.

282.2 MORPHOGENESIS OF SEGMENT-SPECIFIC INNERVATION PATTERNS IN AN IDENTIFIED LEECH NEURON. J. Jellies, C.M. Loer and W.B. Kristan, Jr., Department of Biology, B-022, U.C.S.D., La Jolla, CA, 92093.

Although most of the midbody segments of the medicinal leech (Hirudo medicinalis) are essentially identical, segments 5 and 6 are specialized for reproduction and contain the male and female tissue. Many of the neurons in these segments are correspondingly specialized and hence different from those in the more "standard" ganglia. We have been examining the morphogenesis of one such pair of neurons, the serotonergic Retzius cells, in order to gain insight into the mechanisms by which identified neurons acquire their particular innervation patterns.

One example of a segment-specific difference is that the standard neurons project central axons to adjacent ganglia while those in segments 5 and 6 have no such projections. However, these axons are initiated early in embryogenesis, but later cease extending (A. Mason, J. Glover and W. Kristan, 1984, Soc. Neurosci. Abst., 10:1033). A second obvious difference in Retzius cells is that in the 5th and 6th segments they innervate reproductive tissue, but not body-wall as they do in the standard segments. We used intracellular injections of Lucifer Yellow in embryonic Hirudo to determine whether the peripheral axons in segments 5 and 6 also followed the standard pattern initially, and whether contact with reproductive tissue was correlated with the development of morphological differences.

We found that all Retzius cells extend peripheral axons into the body-wall for almost 72 hours after the processes are initiated. This involves a primarily lateral growth along apparently identical trajectories until late in the 9th day when the axons in segments 5 and 6 come into close association with the reproductive tissue. From that point on, the processes in these segments grow medially to acquire their characteristic patterns while those in standard segments continue lateral growth into the body-wall. The timing of target contact is well correlated with the initiation of changing innervation patterns and the other segment-specific morphological differences in these cells. Thus, we hypothesize that contact with the reproductive tissue signals the neurons in segments 5 and 6 to change their morphogenetic patterns. The predictions of this hypothesis are currently being tested.

This work was supported by grants from NIH.

282.3 THE POSSIBLE ROLE OF TARGET INTERACTIONS IN THE DEVELOPMENT OF SEGMENT-SPECIFIC DIFFERENCES OF AN IDENTIFIED NEURON. C.M. Loer, J. Jellies, and W.B. Kristan, Jr.. Dept. of Biol., U.C., San Diego, La Jolla, CA 92093.

We are interested in the mechanisms by which a neuron assumes its unique identity during development. Segmentally homologous neurons often acquire very different properties, perhaps due in part to the unique segmental environments in which they develop. In standard midbody segments of the leech, Hirudo medicinalis, the large serotonergic Retzius cells innervate the entire skin and body musculature. In contrast, the Retzius cells in segments 5 and 6 do not innervate the body wall; rather, they heavily innervate the male and female reproductive organs found only in those segments. In addition, Retzius cells in segments 5 and 6 differ from all other Retzius cells in soma size, interganglionic branching pattern, neuropilar arborization, and connectivity. We and others (A. Mason and J.G. Glover, submitted) have found that the early development of the Retzius cells in all segments is similar. Differences in the morphology of Retzius 5,6 arise only after their growth cones appear to contact the developing reproductive tissue. At this time, the cells stop extending neurites both laterally into the body wall and into the intersegmental connectives, appearing rather to devote their entire peripheral innervation to the developing reproductive tissue. These observations suggest that interaction with the reproductive tissue is important in the development of the unique characteristics of the Retzius cells in segments 5 and 6.

We are testing this hypothesis with manipulations of leech embryos to ask two questions:

1. Can Retzius 5,6 become like standard Retzius cells in the absence of reproductive tissue? To address this question, we will have ablated the reproductive primordia early in development, before the Retzius cells have contacted the tissue.
2. Can Retzius cells from other segments be induced to become like those in segments 5 and 6? We have addressed this question with two different experiments. We have ablated the Retzius cells in 5 and 6 to see whether Retzius cells from adjacent ganglia, which normally innervate the body wall in these segments, are capable of innervating the reproductive primordia. We have also transplanted reproductive primordia into other segments of the leech embryo. Preliminary results indicate that the Retzius cells of other segments are capable of making a heavy innervation of the reproductive tissue and that this tissue is attractive for Retzius cell processes.

We are continuing experiments to ask whether the development of other unique characteristics of Retzius 5,6 is brought about by interaction with the reproductive tissue.

282.4 HOMOLOGS OF THE HEART ACCESSORY NEURONS IN THE LEECH ARE REVEALED BY DYE FILLING AND ANTIBODY LABELING. Wei-Qiang Gao*, R.R. Stewart and E.R. Macagno (SPON: S. DeRiemer). Dept. of Biological Sciences, Columbia University, New York, NY, 10027.

Several identified neurons, such as the Lateral and Rostral Penile Evertors of Zipser (J. Neurophysiol. 42:455, 1979) or the Heart Accessory (HA) cells of Calabrese & Maranto (J. Comp. Physiol. A154:393, 1984), seem to exist only in the sex segmental ganglia (SG5 and SG6) of the leech nerve cord. Since these ganglia have several hundred more neurons than other SG (Macagno, E.R., J. Comp. Neurol. 190:283, 1980), it is possible that the above-mentioned identified neurons arise uniquely in these SG. Alternately, other ganglia could have homologs of these cells at some point in development, and these later degenerate, or homologs remain but assume different identifies.

Our examination of the HA neurons in SG5 and SG6 [HA(5) and HA(6)] and cells in similar positions within adjacent ganglia in embryos and in adults using dye-filling with Lucifer Yellow and immunocytochemistry with the monoclonal antibody Laz1-1 (kindly provided to us by Dr. Birgit Zipser) lends support to the last of these alternatives. The branching patterns of HA(5) and of HA(6) are very similar in that both have primary processes that extend across the midline and branch into the contralateral roots, but they differ in that HA(5) extends a thick process into the contralateral anterior connective while HA(6) does the same but in the posterior direction. These characteristic branching patterns were seen in the adult and in embryos 20 to 30 days old. Dye fills of cells in very similar positions in adjacent SG revealed cells in SG4 and SG7 with morphologies that closely resemble those of the HA neurons. These cells were designated cells 158 based on their position on the generally accepted map of the leech ganglion. In both embryos and adults, cells 158 in SG4 show the same branching pattern as the HA neurons in SG6, while cells 158 in SG7 look like the HA neurons in SG5.

Cells 158 were also found to label with the mAB Laz1-1, whereas the HA neurons did not. This was shown by double-labeling cells 158 and HA neurons with Lucifer Yellow and Laz1-1 in embryos and adults. In addition, HA neurons of adults label with FMRFamide antibody, but cells 158 do not (J. Kuhlman & R. Calabrese, personal communication.

Developmentally, cells 158 and HA neurons initially share similar branching patterns, extending neurites in both directions along the connective nerves. As development proceeds, however, specific branches of these neurons become large whereas others cease to grow or are eliminated, giving rise to the asymmetric branching seen in the connectives. This observation, the similarity in branching pattern and the similarity in cell body position lead us to postulate that cells 158 and the HAs are homologs. (Supported in part by NIH Grant NS-20336.)

282.5 DIFFERENTIATION AND DEATH OF CELLS IN THE LEECH CNS. I. THE BIPOLAR CELLS. R.R. Stewart and E.R. Macagno. Dept. of Biological Sciences, Columbia University, New York, NY 10027.

To help assess how the adult complement of neurons is achieved during the development of individual segmental ganglia (SG) in the leech CNS, we are examining the antibody labeling of individual cells in embryonic and postembryonic developmental stages of hirudinid leeches. The monoclonal antibody Laz1-1, kindly provided to us by Dr. Birgit Zipser, was raised in mice against the adult nerve cord of Haemopis. It labels between 20 and 35 cells per SG of the adult nerve cord and labels fewer neurons within the sex ganglia (SG5 and SG6) than it does in adjacent ganglia, e.g., SG4 or SG7, in both adults and in embryos. The differences in labeling pattern appear as early as 12 days of development at 24 °C. However, when embryos are labeled between 7 and 12 days, a pair of cells not seen in older embryos is labeled in many ganglia. These cells are medially located on the dorso-posterior aspect of each ganglion. Since two processes appear to project from each cell, one anteriorly and the other posteriorly along the ipsilateral side of the ganglion, we have named these the bipolar cells. Their processes travel within what seem to be well-defined longitudinal tracts.

Bipolar cells are the first to label with Laz1-1, labeling initially in more anterior ganglia (at 7 days), and gradually over the next two days in more posterior ganglia (a new pair begins to label every approximately three hours). By 9 days, when bipolars are labeled in nearly all 32 ganglia, those in the anterior SG , but not those in the head ganglia, begin to die. The first sign of degeneration is the loss of labeling from the anterior and posterior processes. As degeneration of a cell continues, its intensity of labeling decreases in comparison to other bipolars judged to be healthy, and the cell is seen to fragment. Interestingly, the fragments of the dying cell still retain some immunoreactivity as they gradually disappear. As development proceeds, bipolar cells begin to die in more posterior segments as well as in the head, and by 12 days all the bipolars have degenerated.

The function of the bipolar cells is not known, although their early appearance during ganglion formation suggests that they might be responsible for establishing the first longitudinal tracts within the CNS. It is also unknown at present whether these are glial, neuronal or muscle cells. Their degeneration, however, occurs after they have begun to differentiate and during a period in which cell number is decreasing in the SG from an initially higher value than is found in the adult.

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282.6 DIFFERENTIATION AND DEATH OF CELLS IN THE LEECH CNS. II. THE UNPAIRED MEDIAL SEROTONERGIC NEURONS. E.R. Macagno, R.R. Stewart and D. Spergel*. Dept. of Biological Sciences, Columbia University New York, NY 10027.

Of the four types of serotonergic neurons found in the majority of the segmental ganglia of the leech, three are paired (Retzius, Dorso-lateral and Ventro-lateral cells) and one is unpaired (the medial or M cell) in all but a few of the most anterior ganglia. In early embryogenesis the M cells first appear as a pair in each ganglion, as seen in Helobdella by glyoxylic acid induced fluorescence of monoamines (Duncan Stuart, personal communication) and in Haemopis and Hirudo by serotonin antibody labeling (this antibody was kindly provided to us by Dr. Jean Lauder). Is it the case that one M cell simply stops producing serotonin, so that it can no longer be detected with the antibody, or does one of the M cells degenerate in those ganglia where a single M cell is found?

We have obtained evidence in hirudinid leeches which indicates that, in each ganglion with a single M cell, one cell of the original pair dies. As do the bipolar cells discussed in the preceding abstract, the degenerating M cells break appart into many fragments which retain immunoreactivity. Eventually no trace of the cell is seen. In Hirudo, the species that we have examined in more detail, one or two M cells are seen labeling at 9 days of embryogenesis from the first segmental ganglion (SG1) (we did not examine the head ganglia) to between SG5 and SG12. At later times both members of the pair label in all SG. Observations on seven embryos of 11 to 13 days of age revealed that M cells become unpaired gradually from anterior to posterior, with as few as one and as many as seven dying cells observed in individual embryos. By 20 days all SG, with the exception of SG1 and SG2, possess unpaired M cells. Among those cells that were clearly assignable to one side or the other of the midline (some sit right on the midline), as seen in six embryos aged 12 to 20 days, 39 cells were on the left side and 40 on the right side (data pooled for all embryos). Thus, which M cell dies appears to be randomly determined. A possible mechanism of M cell death is competition for the same synaptic targets, with the loser being eliminated. Support for this hypothesis has been provided by the observation (Duncan Stuart, in preparation) that, in Helobdella, killing of the N teloblast, which eliminates all the M cells that arise on the same side, results in the survival of all the M cells generated by the teloblast on the other side.

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282.7 HORMONAL REGULATION OF POSTEMBRYONIC NEUROGENESIS IN THE MOTH MANDUCA SEXTA. R. Booker* and J. Truman (SPON: M. Murray). Univ. of Washington, Seattle, Wash. 98195

All the segmental ganglia of M. sexta larvae contain neuroblasts, which generate discrete clusters of partially differentiated neurons, the preganglion cells (PGCs). During metamorphosis a wave of cell death sweeps through the clusters eliminating 10-100% of the PGCs. At about the same time the surviving PGCs begin the process of terminal differentiation to mature neurons. Manduca's postembryonic development is controlled by a few developmentally important hormones. Using a combination of histological and endocrine techniques, the developmental regulation of postembryonic neurogenesis in Manduca by the steroid hormone ecdysone was examined.

Larvae were ligated between the thorax (the source of ecdysone) and the abdomen during the early phases of metamorphosis. The fourth abdominal ganglia (A4) of the isolated abdomens were then examined at a point corresponding to two days after pupation. The development of the abdomens ligated during the first days of metamorphosis was incomplete; in A4 minimal cell death and differentiation were observed within the clusters of PGCs. When the ligature was applied later in development, both the extent of cell death and differentiation of the PGCs increased. The adult cell number and morphology were observed only if the abdomens were ligated on or after the day of pupation.

A direct effect of ecdysone on neurogenesis was shown by combining ligation with hormonal replacement. Abdomens were ligated at the onset of metamorphosis. The ligated abdomens were then infused with ecdysone and examined six days later. Both cell death and differentiation were triggered in the isolated abdomens in response to the infused ecdysone. The magnitude of the responses observed was dose dependent. While ecdysone may trigger these developmental events the extent of the cell death and the morphology of the mature neurons was found to be cluster specific.

282.8 SEROTONERGIC CELLS IN *Drosophila*. A.M. Valles* and K. White*. (SPON: J. Hall). Dept. of Biology, Brandeis University, Waltham, MA 02254.

The study of the distribution of the neurotransmitter serotonin (5HT) in *Drosophila melanogaster* has been initiated in an attempt toward understanding the extent of the role of this neurotransmitter in the ontogeny of the nervous system. Genetic variants allow one to alter the levels of 5HT and thereby probe the impact of 5HT on neuronal development.

Immunocytochemical techniques have been used to identify 5HT-containing cells in the central nervous system (CNS) (White and Vallés, *Molecular Bases of Neural Develop.*, Ed. G.Edelman, W.Gall and W.Cowan, 1985). In the present study, we extend the localization of 5HT immunoreactivity during development and demonstrate that a) the onset of staining of 5HT cells appears in the CNS during late embryogenesis, b) the 5HT stereotypic pattern persists post-embryonically and c) additional post-embryonic cells appear during pupation. In the neuropilar region, extensive immunoreactive processes and axonal arborizations are observed. The larval nervous system shows peripheral 5HT immunoreactive innervations to the ring gland and the cephalopharyngeal muscles.

In *Drosophila*, the gene *Ddc* encodes the enzyme dopa decarboxylase (Wright et al, Genetics,84:287, 1976) which catalyzes the decarboxylation of 5HTP to 5HT as well as l-dopa to dopamine (Livingstone and Temple, Nature, 303:67,1983). The effect of *Ddc* mutations on 5HT cells was studied by deleting the *Ddc* gene. *Ddc* deficient animals can survive until early pupal period. The CNS of the *Ddc* deficiency larvae were characterized by a complete absence of immunoreactive cells. However, 5HT neurons are still present since incubating the tissue in exogenous 5HT results in the reappearance of the immunoreactive pattern. Work is currently being conducted to determine the effect of *Ddc* mutations on dopaminergic cells (see Budnik et al, this volume). (Supported by NIH Grant GM31503).

282.9 DEVELOPMENT OF AMINE AND PEPTIDE NEURON SYSTEMS IN EMBRYONIC AND LARVAL LOBSTERS-E. Kravitz, B. Beltz and K. Siwicki* Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115

We have begun a study of the appearance of the amines, serotonin and octopamine, and the peptide proctolin, in embryonic and larval lobsters. The aim is to examine when in development identified neurons first express these substances, what controls this expression, and when these neurons first become functional, and then to correlate these events with the emergence of aspects of lobster behavior.

Female lobsters carry on their swimmerets between 10,000 and 100,000 eggs,all fertilized within a few days of each other. Embryonic development is slow(up to 9 months in the wild), but can be speeded up by keeping eggs in warm water.The first stage, free-swimming larvae, have no claws, walking legs or swimmerets, are top-dwellers that move towards light and show no "hiding" behavior. 2 to 3 weeks and 3 molts later,4th stage larvae hatch that resemble miniature lobsters.They descend to the bottom, burrow and hide, and begin the solitary existence of young lobsters.4th stage larvae swim in a fully extended posture, similar to one induced in older animals by injection of octopamine.

We have mapped the patterns of immunocytochemical staining for the 3 substances in animals half-way through embryonic development, in later embryos, and in various larval stages. Staining for serotonin appears early in development (see Taghert and Goodman, J. Neurosci.4,989) and the full complement of neurons staining for serotonin in adults can be identified in the earliest embryos we examined. The serotonin-staining neurons are among the largest cells in the embryo. A pair of brain cells are about 30$\mu$m in diameter in early embryos and about 60$\mu$m at hatching. The identified serotonin-proctolin cell pairs in the T5 and A1 ganglia of adult lobsters (Beltz and Kravitz, Soc. Neurosci. Abstr.10,152) also are easily found in embryos. They are large (15$\mu$m) neurons found in characteristic midline locations and their major neuropil branches can be recognized in early embryos. In contrast proctolin staining (seen in about 1500 neurons in adult lobsters) is observed in only a few hundred cell bodies in embryos, mainly in regions of the nervous system that regulate feeding. During the lst to 4th larval stages, staining appears in the remaining neurons. This includes the T5-A1 serotonin-proctolin neurons, which therefore express their amine and peptide functions at widely different times in development. In certain neurons, staining for proctolin is seen only in embryos. Preliminary studies with an antibody to octopamine show a proctolin-like pattern of staining: well stained neurons only in the anterior part of the nervous system in embryos, and an adult-like pattern by the 4th larval stage. Biochemical studies to complement the immunocytochemical investigations are presently under way. (supported by the Klingenstein Fund and NIH)

282.10 COMPETITION CONTROLS QUANTAL RELEASE AT AN IDENTIFIED INSECT SYNAPSE. D.Shepherd* and R.K.Murphey Neurobiology Research Center, SUNY Albany, Albany, NY 12222.

A small group of sensory hairs on the cricket cercus are innervated by neurons (x-neurons) that terminate in both the ipsilateral and contralateral halves of the terminal abdominal ganglion. Recent anatomical studies have demonstrated that long term removal of the left cercus causes a distortion of the remaining right, x-neuron, it adds arbors on the side of the amputation and decreases the number of arbors on the side opposite to the amputation (Murphey & Lemere.Science.224. 1352-1355.1984).

Simultaneous recording from 2 bilaterally homologous interneurons and the right x-neuron have demonstrated that following deafferentation the synaptic connections of the x-neurons are reorganised. In untreated animals the right x-neuron produces a monosynaptic e.p.s.p. in both medial giant interneurons (MGI). In unilaterally deafferented animals, the x-neuron fails to produce a detectable e.p.s.p. in the right MGI, suggesting that as a result of deafferentation the x-neurons have retracted synapses from the right MGI. In the left MGI the same x-neuron produces an e.p.s.p. twice the size of the e.p.s.p. seen in normal animals, indicating an increased efficacy of the synaptic connection. These changes in connectivity are entirely consistent with the anatomical changes seen in the x-neurons.

Using quantal analysis of the inputs to the left MGI it has been possible to further examine the presynaptic changes produced by the reorganisation of the x-neuron. Using binomial statistics to determine m, the mean quantal content, it was demonstrated that m=0.9 in untreated animals and m=2.37 in experimental animals. This increase in m indicates that a change in the presynaptic neuron contributes to the enhanced contact and is correlated with the shift of the x-neuron terminals in the region near MGI. Further, the binomial product n in experimental animals (n=6.3) is nearly twice that seen in normal specimens (n=3.14). Recent studies have suggested that n is directly correlated with the number of contacts between pre and postsynaptic neurons (Korn et.al.,Science.213, 898-901.1981). Preliminary light microscope studies of our material suggest that the number of contacts is low (fewer than 10) in control animals and is increased in treated specimens. In conclusion, the removal of neighboring presynaptic cells alters the structure of neuron-x and this has a direct correlate in changes of synaptic efficacy.

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282.11 SYNAPTIC PLASTICITY IN RESPONSE TO PARTIAL DEAFFERENTATION OF GIANT INTERNEURONS IN THE AMERICAN COCKROACH. Susan Volman. Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

The giant interneurons (GI's) of the cockroach escape system receive input from a bilateral pair of sensory organs, the cerci, which contain an array of wind-sensitive sensilla. Within one month after unilateral cercal lesions, GI's which received their major input from the ablated cercus become more responsive to stimulation of the remaining cercus. This enhanced responsivity underlies functional plasticity in the escape behavior which also occurs after unilateral cercal ablations (Vardi and Camhi, J. Comp. Physiol. 146: 299, 1982).

In order to study the physiological basis for enhancement, recordings were made from the GI somata which are located across the ganglionic midline from the axons. Small dendrites arise ipsilateral to the soma, whereas the major dendrites and integrating zone are ipsilateral to the axon. The size and shape of compound EPSP's, as well as recordings from GI's in ganglia which had been split along the midline, showed that each set of cercal inputs connects mainly ipsilaterally to areas of the neuropil which are about 100μm apart. After long-term ablations of the major input, there was no indication that the location of connections from the intact cercus had changed, although this input was now more likely to evoke action potentials. There was also no evidence for any changes in the cable properties of the GI's.

I next looked for changes in unitary EPSP's evoked by stimulation of single afferents. The filiform hairs on the cerci are arranged in distinct rows and columns and each hair is associated with a single sensory neuron. Thus it is possible to reidentify single afferent neurons from one animal to the next. I confined this study to 4 afferents (hairs 3-6 from column h on the cercus ipsilateral to the soma) which make a large contribution to the receptive fields of GI's 1 and 2 after ablation of the contralateral cercus. Unitary EPSP's, correlated one-to-one with action potentials in individual afferents, were recorded from 8 intact animals and 6 animals at least 30 days after unilateral cercal ablation. For GI 2, these EPSP's were significantly larger in the deafferented animals, averaging 4.3mV as compared to 1.8mV in the intact animals. For GI 1, however, there was no significant difference between the size of EPSP's in the two groups, perhaps because these hairs play a less important role in the enhanced response of this GI.

Cobalt fills of column h afferents showed that they arborized purely ipsilaterally. Thus the physiologcal results for GI 2 suggest that, after partial deafferentation, enhanced responsiveness may be accounted for by increased synaptic current at a site widely separated from the locus of deafferentation.

282.12 ADIPOKINETIC HORMONES IN LOCUSTS: SYNTHESIS AND DEVELOPMENTAL REGULATION: S. Hekimi* and M. O'Shea. Lab. of Neurobiology, Univ. of Geneva, CH-1211 Geneva 4, Switzerland.

Adipokinetic hormone (AKH) is a sequenced decapeptide (pGlu-Leu-Asn-Phe-Thr-Pro-Asn-Trp-Gly-Thr-$NH_2$) which functions in adult locusts (Schistocerca sp. and Locusta sp.) to mobilize lipids during flight (see Stone and Mordue in "Neurohormonal Techniques in Insects" Ed. T.A. Miller, Springer Verlag, 1980). It is found in high amounts (~ 500 pmol) in the corpus cardiacum (CC), a major insect neurosecretory structure. The CCs of locusts also contain a second AKH-activity (AKH II). The reported amino acid (a.a.) compositions of AKH II in Schistocerca and Locusta differ, whereas the structure of AKH I is the same. AKH II is related to AKH I (Carlson, Insect Biochem 9, 1979). A proposed sequence for Schistocerca AKH II is pGlu-Leu-Asn-Phe-Ser-Thr-Gly-Trp-$NH_2$. The a.a. composition of Locusta AKH II suggests that $Thr^6$ is substituted with Ala (Gäde, G. Hoppe-Seyler's Z. Physiol. Chem. 365, 1984). By incorporating tritiated amino acids into in vitro cultured CCs and by reverse phase HPLC we confirm that the two AKH IIs are different and that the a.a. composition of Schistocerca AKH II is correct. Using the in vitro system we can $^3H$ label AKH I and II to high specific activity (order of 1Ci/mmol/24 hr culture). We are currently using this method to study AKH biosynthesis and its regulation. It may also allow for receptor identification and for establishing RIAs that distinguish between AKH I and II.

Using molecular sieving and reverse phase HPLC we have isolated a CC protein ("X") that may be an AKH precursor. It has a molecular weight ≤10 Kd (AKH = 1 Kd). In in vitro culture "X" incorporates all the a.a.s present in AKH. It is the only compound to do so at a rate comparable to that for AKH. As expected for a precursor, "X" does not accumulate in the CC and contains Arg and Lys residues.

To study developmental regulation we have measured AKH I and II levels in different stages and sexes. There is a gradual but dramatic shift in the ratio of AKH II/AKH I from the adult (ratio ~0.2) to near equality in the late embryo or first instar (ratio ~ 0.8). From the 2nd instar on, the AKH ratio shows consistent sexual dimorphism; the female having the slightly higher ratio. Absolute amounts of AKH I and II appear not to change even normalized to body weight, except in adults. During adult maturation a steep increase of AKH I and II occurs and a difference between males and females by a factor of about 2. The presence of both AKH peptides throughout development and their differential regulation suggest functional roles not solely related to flight, an entirely adult behavior. Supported by E.I. du Pont de Nemours and Company.

282.13 TRANSNEURONAL INDUCTION OF MUSCLE ATROPHY IN GRASSHOPPERS. Mark Weidner*and Edmund A. Arbas. (SPON: E.Lasater).Northeastern University, Boston, and Biological Laboratories, Harvard Univ. 16 Divinity Ave. Cambridge, MA. 02138.

Autotomy is a process evolved in a number of animals whereby nonessential body parts are shed for defense or to abandon a damaged extremity. Grasshoppers and locusts often drop a jumping leg by autotomizing between the 2nd and 3rd leg segments (trochanter and femur). This severs branches of the leg nerve (N5), but does not damage muscles, as none span this joint. We find in the grasshopper, Barytettix psolus, that muscles intrinsic to the thorax that operate the coxa (1st leg joint) and that are neither damaged nor denervated by autotomy, atrophy to less than 15% of their normal mass following autotomy. Atrophy is localized to the side and body segment where autotomy occurs. Atrophy begins about 10 days after loss of a limb, is complete by about 30 days, and follows a similar timecourse whether induced in young adults or sexually mature grasshoppers.

In addition to severing N5, hindlimb autotomy reduces load on the coxal muscles, and alters coxal use in behavior, possibly via changes in motor outflow to its muscles. We have tested the possible contributions of these influences to muscle atrophy by: 1) cutting N5 near the metathoracic ganglion without inducing autotomy, 2) loading the coxal muscles after autotomy by re-attaching the limb (weighted to compensate for dehydration) in its natural position, 3) inducing abnormal postures of the coxa by cutting the tendon of the tibial extensor muscle and causing abnormal leg use during behavior. All procedures severing N5 caused maximal atrophy. Re-loading coxal muscles did not significantly prevent atrophy. Abnormal use of the leg did not result in any significant atrophy of coxal muscles. We conclude that the peripheral severing of the leg nerve that occurs during autotomy is the primary cause of atrophy of the coxal muscles. Neurons with projections in N5 thus appear to affect central neurons that separately innervate coxal muscles and through them induce atrophy. This effect may occur through degeneration or atrophy in the CNS of neurons projecting in N5, or through the alteration of their physiological activity and the subsequent influence of these changes on neurons innervating coxal muscles. Preliminary anatomical studies in which selected coxal muscle motorneurons were stained by backfilling with cobalt show no major changes in motorneuron morphology that accompany muscle atrophy and thereby point to changes in the physiological or biochemical activity of these neurons as the determinant of muscle atrophy.

We thank R.L. Calabrese for providing materials and facilities for this study.

283.1 ON THE ORDER OF 10,000 CONSTRAINTS ON NEURAL CONNECTION VALUES MUST APPARENTLY BE SATISFIED TO ENABLE CERTAIN ASPECTS OF CONCEPT REPRESENTATION AND PRODUCTION OF SEMANTIC ERRORS. R. Martin. Biochem. Lab., Chemistry Dept., Brooklyn Coll., Bklyn., NY 11210.

Among the most startling of mental errors are those that involve substitution of different concepts for one another. For example, a person who is asked to copy the sentence "Tigers growl" may write instead: "Lions growl." A key feature of such semantic errors is that words which replace one another are almost always related: "lion" and "cat" may replace "tiger" but not "orange" or other semantically unrelated words. Slightly more than 10,000 constraints on permissible connection values within a structurally homogeneous network enable -- and are apparently required for -- this type of mental error to be modelled in a manner that is consistent with other aspects of concept representation. These constraints include those that must be satisfied for novel and familiar examples of familiar categories to be appropriately named, for groups containing members of several categories to be named, for information to be correctly transferred from one concept to another, and for the severity of errors to be minimized when inappropriate names or labels are selected. The model involves only six learning rules that guide adjustment of connection strengths to values that generally satisfy the 10,000 constraints. The model is of interest because of recent experimental observations supporting key assumptions (1) and because the approach taken suggests explanations for a number of other cognitive abilities (2). Connection values are so highly constrained -- with several hundred constraints simultaneously limiting the permissible value of each connection -- that it may not be possible to formulate a qualitatively different, alternative explanation for these aspects of concept representation and production of semantic errors.

1) Levy, W.B. and O. Steward (1983) "Temporal contiguity requirements for long-term potentiation/depression in the hippocampus," Neuroscience 8(4), 791-797.

2) Martin, R., Lukton, A. and S.N. Salthe (1984) "Simulation of simple cognitive maps, concept hierarchies, learning by simile, and similarity assessment in homogeneous neural nets," The Proceedings of the 1984 Summer Computer Simulation Conference, Soc. for Computer Simulation, vol. 2, 808-821.

283.2 FREQUENCY ESTIMATES OF SELF-GENERATED AND EXAMINER-PROVIDED WORDS: EVIDENCE FOR A FRONTAL-LOBE CONTRIBUTION. M.L. Smith* and B. Milner. Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada, H3A 2B4.

The "generation effect" refers to the finding that, in normal subjects, memory for self-generated words is better than memory for words that are externally presented (Jacoby, 1978; Slamecka & Graf, 1978; McFarland et al., 1980). The generation effect is strong across a variety of testing conditions and is independent of the depth of processing of the material. Raye, Johnson & Taylor (1980) extended these findings to frequency judgements, showing that people are more sensitive to the relative frequency of internally generated events than of externally presented events.

Smith (1985), and Smith & Milner (1983) have demonstrated deficits after frontal lobectomy in estimating the number of times words or abstract designs (provided by the examiner) appeared in lists. In view of the robustness of the generation effect in normal subjects, the question arose as to whether the impairments seen in patients with frontal-lobe lesions in sensitivity to frequency of occurrence would also be seen with words generated by the patients themselves.

Patients with excisions from the left frontal (LF), right frontal (RF), left temporal (LT), or right temporal (RT) lobe, and normal control subjects (NC) were given two tasks. In one, they were required to generate words in response to clues, whilst, in the other, they simply had to read responses that satisfied the clues. In both tasks, the target words appeard 1, 3, 5, 7, or 9 times. After generating, or reading, all the target words, the subjects were required to estimate the number of times each word had appeared.

The results indicated that all subjects gave higher frequency estimates overall to the words that they generated themselves than to those they had merely read. This effect did not interact with locus of lesion. However, across both tasks, the LF and RF groups underestimated frequency of occurrence relative to the LT, RT, and NC groups, at the higher frequency levels; the LF group was impaired at frequencies 5, 7, and 9, and the RF group was impaired at frequency 9.

These findings demonstrate a role for both frontal lobes in sensitivity to frequency of occurrence of both internally generated and external events. In keeping with the verbal nature of the test material, there appears to be a stronger contribution by the left than by the right frontal lobe to frequency estimates on this task.

283.3 STRUCTURAL AND FUNCTIONAL ABNORMALITIES OF FRONTAL LOBE IN SCHIZOPHRENIA. K.F. Berman*, R.C. Shelton*, R.F. Zec, and D.R. Weinberger*, Section on Clinical Neuropsychiatry, NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032.

Several observations suggest abnormalities of dorsolateral prefrontal cortex (DLPFC) in schizophrenia. These include clinical symptoms similar to those of frontal lobe disease, decreased relative DLPFC regional cerebral blood flow (rCBF), and animal studies suggesting a role for DLPFC in cognitive processes commonly impaired in patients with chronic schizophrenia. Recent research in schizophrenia has identified two relatively consistent brain abnormalities: 1)structural findings such as enlarged ventricles and cortical atrophy, especially of frontal lobes, on CT scan; and 2)functional abnormality of frontal cortex in studies employing techniques such as regional cerebral blood flow (rCBF) that investigate physiology of the living human brain. These findings of structural and functional abnormalities are potentially important to the understanding of schizophrenia, but it has not been clear whether they are related. The present study combines determinations of cortical function via Xenon133 inhalation rCBF with investigation of structure by computed tomography (CT) scan.

rCBF, an indicator of cortical metabolism, was measured during various cognitive tasks. First, to assess DLPFC function, 20 patients medication-free (DF) for at least four weeks, 24 patients treated with neuroleptic medication, and 25 normal subjects completed a three-test series: rCBF was determined initially during the resting state and then while subjects performed, in counterbalanced sequence, an automated version of the Wisconsin Card Sort (WCS) to selectively test DLPFC, and a simple numbers matching task (NM) to control for non-DLPFC-related aspects of the procedure. Next, to assess the roles of attention and task specificity in DLPFC function, rCBF was measured in 17 DF patients and 18 controls during two versions of a visual continuous performance task (CPT), an attentional task not specific for DLPFC. Finally, to further determine DLPFC function during complex, but non-DLPFC-linked, reasoning, rCBF studies were done while subjects solved Raven's Matrices (RM). Brain structure of 20 DF and 22 medicated patients who completed the WCS/NM paradigm was assessed with CT.

DLPFC rCBF, specifically, was lower in DF patients compared to normals during the WCS. Treatment with neuroleptic medication did not reverse this DLPFC inactivity. In contrast, during the non-DLPFC-specific CPT's, NM, and RM tasks, no difference in DLPFC blood flow between patients and controls was noted. Degree of DLPFC activation during the WCS was correlated with patients' performance on this task (r=.52, p .02) but not with their degree of autonomic arousal during the procedure. DLPFC rCBF correlated with several parameters of structural pathology in CT, including frontal atrophy and lateral ventricular size.

These data suggest a potential pathophysiological mechanism for the cognitive impairment in schizophrenia. These results also indicate that physiological observations of reduced DLPFC rCBF are related to structural pathology, that these abnormalities are linked, and that they are important biological underpinnings of this illness.

283.4 ALTERATIONS OF LOCAL CEREBRAL GLUCOSE METABOLISM IN MOOD DISORDERS. M.E. Phelps, L.R. Baxter, J.C. Mazziotta, J.M. Schwartz. Division of Nuclear Medicine and Biophysics, UCLA School of Medicine.

Studies of local cerebral glucose memtabolic rate (LCMRGlc) with (F-18) fluorodeoxyglucose (FDG) were performed with positron emission tomography (PET) in patients with unipolar depression (n=11), bipolar depression (n=8), hypomania (n=8), bipolar mixed states (n=3) and excessive compulsive disorder in depressed and non depressed states (n=5). Studies were carried out in drug free states as well as during spontaneous and drug induced changes in mood. Age/sex matched normals (n=12) were also studied. All studies were carried out in ambient ears/eyes open resting state. Major findings were: 1) bipolar depressed patients had lower ($p < 0.001$) supratentorial CMRGlc (16.7±1.3 SEM), than normals (23.6±0.7), hypomanic bipolar (24.7±1.6) or unipolar depressed (24.5±0.9). Values in parenthesis are CMRGlc in units of μmoles/min/100gms. Bipolar mixed patients had supratentorial CMRGlc values (16.4±2.8) that were not different from bipolar depressed patients but were lower than all other groups ($p < 0.02$). Bipolar depressed and mixed states showed increased (31%) supratentorial CMRGlc ($p < 0.02$) with elevated mood (euthymic or hypomanic). Three rapid cycling bipolar patients also showed consistent increases (35%) in supratentorial CMRGlc from depressed to elevated mood state. The agreement of CMRGlc in paired studies of the depressed state in 2 of these patients was ±8%. Unipolar depresssed patients had a low LCMRGlc ratio of caudate to hemisphere (Cd/Hem) (1.18 ±0.03 SEM) compared to bipolar depression (1.3 ±0.4) or normals (1.32 ±0.02). Four unipolar patients studied after drug induced recovery all showed corresponding return of Cd/Hem ratio to value in normals. Patients with excessive compulsive disorder had significant hypometabolism in the left frontal and anterior cingulate during the depressed state that return to normal levels and left/right symmetry in the non depressed state. Results of these studies show 1) delineation of bipolar depressed from unipolar depressed patients and normals by changes in supratentorial CMRGlc but no apparent focal changes in bipolar depression, 2) seperation of mixed bipolar from unipolar depression, 3) delineation of unipolar from bipolar depression and normals by focal changes in the caudate, 4) identification of focal (frontal and cingulate) changes in LCMRGlc in excessive compulsive /depressed state and 5) direct correspondence of changes in CMRGlc and mood state in these patients independent of whether changes in mood were drug induced or spontaneous.

283.5 EQUIVALENT PATTERN OF ORDER MEMORY PERFORMANCE IN RATS WITH NUCLEUS BASALIS MAGNOCELLULARIS LESIONS AND HUMANS WITH ALZHEIMER'S DISEASE. R. P. Kesner, K. A. Crutcher, and T. B. Adelstein* (SPON: S. W. Miller), Dept. of Psychology, Univ. of Utah, Salt Lake City, Utah 84112.

It has become of increasing importance to develop an animal model of mnemonic symptomatology associated with Alzheimer's disease in order to understand its underlying neuropathology and to develop therapeutic methods aimed at alleviating memory symptoms. In order to develop such a model, rats with ibotenic acid lesions of the nucleus basalis magnocellularis (NBM) were tested in an order memory task for an 8-item list of varying spatial locations within an 8-arm radial maze. Humans diagnosed as having Alzheimer's disease as well as healthy elderly subjects were also tested in an order memory task for a 6-item list of varying spatial locations with X's placed on a single sheet of paper containing twelve possible locations. The critical procedures used to test the NBM lesioned animals were identical to that used to test Alzheimer's patients. For each trial a list of pseudo-randomly selected locations was presented. After presentation NBM lesioned animals or Alzheimer's disease patients were given a single test with a choice between two locations (arms in maze or X's on a sheet of paper). The rule to be employed was to choose the location (arm or X) that ocurred earlier in the presentation sequence.

Rats with small NBM lesions resulting in small AchE depletion of parietal cortex and parts of frontal cortex had normal performance for the first, but impaired performance for the last choice orders of the list. Animals with large NBM lesions resulting in large AchE depletion of parietal and part of frontal cortex displayed an order memory deficit for all choice orders of the list.

Patients with an diagnosis of early Alzheimer's disease compared to healthy elderly subjects also had normal performance for the first, but impaired performance for the last choice orders of the list. This pattern of results is identical to what is seen in rats with small NBM lesions. Patients with diagnosis of late Alzheimer's disease, who can still perform the task, display an order memory deficit for all the choice orders of the list . This result is identical to what is seen in rats with large NBM lesions. These data suggest that perhaps early damage to NBM area in humans might be critical in producing specific memory deficits. Furthermore, these data provide a basis for a possible animal model of the mnemonic symptomatology of Alzheimer's disease.

283.6 RECALL AND CLUSTERING BY ALCOHOLIC KORSAKOFF PATIENTS. D. Kyaw*, R. H. Bauer and M.M. Kilbey. Dept. of Psychology, Middle Tennessee State University, Murfreesboro, TN 37132.

Alcoholic Korsakoff patients (KP) are severely impaired in remembering events in the immediate past (minutes) and have difficulty learning new information. Normal learners identify organization in verbal material and use this knowledge to increase recall. For example when presented with lists made up of words from several categories or random word lists, normal learners recall more category words and recall more words consecutively from the same category (clustering), even though the category words are presented at random. Recall of category lists is higher than random lists in both KP and alcoholic controls (AC), but recall of both list types is lower in KP than AC, even though clustering by KP and AC is not significantly different. Since recall of category lists and the level of clustering are positively related in normal learners, it is surprising that recall of category lists is lower in KP than AC, but clustering by KP and AC is no different. The major purpose of the present study was to further examine recall and clustering of category words and to extend this area of research by comparing recall and clustering of rhyming and associated words.

The subjects were KP, AC, and nonalcohlic controls (NAC) that were matched for age, sex, education, and IQ (n = 9 per group). Each subject was given 8 trials in which 9 words each were presented at the rate of one word per 2 sec. Each list was composed of 3 rhyming words, 3 associated words, and 3 category words. Immediately after the last word of each list, the subjects recalled the words in any order.

Statistical analyses showed that KP recalled significantly fewer rhyming, associated, and category words than the other two groups, but recall of each word type was comparable in AC and NAC. Both AC and NAC recalled significantly more associated and category words than rhyming words, but KP recalled a comparable number of rhyming, associated, and category words. There were no significant differences among the groups in clustering of the three word types and clustering of each word type was comparable in the three groups.

The finding that recall of category words is lower in KP than AC but clustering is no different is in accord with previous findings. The present study shows, in addition, that recall of rhyming and associated words by KP is lower than normal learners, but clustering of rhyming and associated words is not lower in KP than normal learners. These findings suggest that the lower recall of verbal material by KP is not due to inadequate organization of the material.

283.7 MEMORY AWARENESS (METAMEMORY) FOR PERFORMANCE IN A MEMORY TASK BY ALCOHOLIC KORSAKOFF PATIENTS. R.H. Bauer, D. Kyaw*, and M.M. Kilbey Department of Psychology, Middle Tennessee State Univ. Murfreesboro, TN 37132.

For the last century there has been a debate concerning the degree to which alcoholic Korsakoff patients (KP) are aware of their own learning and memory abilities and characteristics. Some researchers maintain that KP are aware of their memory problems but attempt to consciously conceal their deficits by fabricating tales to fill in memory gaps in order to spare themselves embarrassment, i.e. KP confabulate. Others have suggested that KP are unaware that they suffer from a severe memory disorder and are in no position to decide whether their replies are correct or incorrect. The major purpose of the present study was to provide information concerning awareness of KP's own memory abilities and strategies for a memory task they performed.

The subjects were KP, alcoholic controls (AC), and nonalcoholic controls (NAC) (n = 9 per group) that were matched for age, sex, education, and IQ. Each subject was given 8 trials in which lists of 9 words each were presented by a slide projector. The words were presented at the rate of one word per 2 sec, and immediately after the last word the subjects recalled the words in any order. Immediately prior to one half the trials the subjects were asked to estimate the number of words they thought they could recall on that trial. After being tested in the recall task, the subjects were asked questions concerning what they had been doing to recall the word lists. The first questions were open-ended or required examples, whereas later questions provide cues for a desirable response.

As compared to AC and NAC, KP recalled fewer words and over estimated the number of words they thought they could recall, suggesting that KP are less aware of their own learning and memory abilities. Since the degree of correspondence between open-ended questions which provide no cues for correct replies and questions providing cues for a desirable answer is no worse in KP than AC and NAC, it appears that KP were not intentionally hiding their poor learning strategies. Comparison of performance in the memory task and answers on the questionnaire indicated that KP were unaware of appropriate strategies to use in the memory task and that KP answered the questionnaire in a manner which corresponds to how they actually perform the memory task. In general, these findings indicate that although KP over estimate their memory abilities, they are aware of their learning and memory strategies, and do not attempt to conceal their poor strategies.

283.8 DBI, A PROCONFLICT NEUROPEPTIDE PURIFIED FROM RAT BRAIN IS FOUND IN HUMAN BRAIN. P. Ferrero*, A. Guidotti, B. Conti-Tronconi and E. Costa (SPON: S. H. Koslow). Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hospital, Washington, D.C. 20032 and Div. Chemistry and Chemical Engineering, Cal. Inst. Technol., Pasadena, CA 91125.

DBI is a neuropeptide purified from rat brain homogenates which displaces beta-carbolines from specific brain binding sites. When injected intraventricularly in rats DBI has a pharmacological profile similar to that of anxiogenic beta-carbolines. Studies with tryptic DBI fragments suggest that the active portion of DBI molecule is contained in an octadecaneuropeptide (ODN) with the following amino acid sequence: Gln-Ala-Thr-Val-Gly-Asp-Val-Asn-Thr-Asp-Arg-Pro-Gly-Leu-Leu-Asp-Leu-Lys (Neuropharmacology 23: 1359, 1984). Working with post mortem human brain extracts, we have now purified a neuropeptide which appears to be similar to rat DBI with respect to molecular weight (approx. 11,000) amino acid composition and retention time on reverse phase HPLC. Similarly to rat DBI, the neuropeptide extracted from human brain elicits proconflict responses when injected intracerebroventricularly in thirsty rats.

Human DBI cross-reacted with an antibody raised against synthetic ODN with a potency similar to that of rat DBI. Moreover by sequencing various tryptic fragments of human DBI, we detected the ODN sequence found in rat DBI. However studying a number of antisera raised in rabbits against human and rat DBI, we found a poor cross reactivity (1/100) between the rat and human neuropeptides. Using a specific antibody raised against human DBI we have measured DBI content in few areas of post mortem human brain and found a regional distribution (amygdala > cerebellum > hippocampus > cortex > striatum) similar to that observed in rat. Moreover DBI-like immunoreactivity was found in spinal fluid of a small group of healthy volunteers. The data suggest that human and rat DBI though similar may not be identical, immunologically. However since similarly to rat DBI, human DBI causes a proconflict action in rats when injected intraventricularly and since they both contain the ODN sequence we believe that human and rat DBI are equivalent functionally. Moreover the presence of high concentrations of DBI in selected areas of human brain and in spinal fluid allows to infer that this neuropeptide may play an important role in the manifestation of anxiety and other symptoms of various neuropsychiatric disorders.

283.9 MEASURING DBI-LIKE IMMUNOREACTIVITY OF HUMAN CEREBRO-SPINAL FLUID. M.L. Barbaccia*, P. Ferrero*, A. Guidotti, D. Pickar*, S. Paul, F.K. Goodwin and E. Costa. Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hospital, Washington, D.C. 20032 and Div.of Intramural Research Programs, NIMH, Bethesda, MD 20814

Human brain contains a neuropeptide (HDBI) (Ferrero et al., Neuroscience Abstracts 1985) very similar to the anxiogenic peptide extracted from rat brain (Science 277:934, 1985). The aim of the present investigation was to assess the reliability in measuring the content of DBI-like immunoreactivity of human cerebro-spinal fluid (CSF). These studies were carried out in a group of 28 normal volunteers age and sex matched with a group of 45 patients suffering from various neuropsychiatric disorders. An antiserum against purified HDBI was raised in rabbits; it detects selectively and with high sensitivity (10 fmole) HDBI in brain extracts and CSF. Only 100 µl of CSF are needed for a reliable quantitation of HDBI-like immunoreactivity. The specificity of this assay was determined by immunoblotting and HPLC. For routine assay CSF was acidified (1 N acetic acid) and 100 µl aliquots of the extract were tested for competition with $^{125}$I-Bolton-Hunter reagent-labeled HDBI. The displacement of $^{125}$I-HDBI by increasing concentrations of CSF extract shows a slope identical to that of authentic HDBI. The HDBI stability in CSF was tested by measuring HDBI like immunoreactivity in aliquots of the same sample after different treatments: 1) heating 15 minutes at 90°C; 2) acidification with 1 N acetic acid and heating; 3) acidification with 0.1 N and 1.0 N acetic acid; 4) freezing and thawing; 5) incubation for up to 24 hrs at room temperature. The highest content of HDBI-like immunoreactivity was found in acidified samples. Serial measurements of HDBI-like immunoreactivity in the same samples yielded virtually the same amounts. Moreover, when serially collected fractions of CSF from normal volunteers were assayed virtually no concentration gradient of HDBI-like immunoreactivity was found. Pooling together the 73 samples, the amount of HDBI-like immunoreactivity in CSF appears to change with age (aged > young) and sex (male > female). We have indications of modifications in CSF-HDBI content related to psychiatric symptoms, but at this time, we refrain to make any conclusion waiting for a greater number of cases to be analyzed.

283.10 HANDEDNESS AND VISUO-CONSTRUCTIVE PERFORMANCE IN HUMANS. P.J. Donovick[1], R.G. Burright[1*], M.E. Kovaleski[1*], D. Bronzino[1*], G.R. Ruhl[1*], and A. Yozawitz[2]. [1]Dept. Psychology, SUNY Binghamton, Binghamton, NY 13901, [2]Neuropsychology Laboratory, Hutchings Psychiatric Center, Syracuse, NY 13210.

The present investigation was designed to address how handedness, assessed with a common laterality scale, would interact with visuo-constructive performance. Right and left handed male and female college students were evaluated for style of visuo-constructive performance on a block design task, including individual preferences for hand manipulation and for starting position of the first block.

Analysis of block design performance revealed that task complexity influenced hand use. For 2x2 patterns, males and females frequently employed bimanual strategies for their constructions. When complexity increased to 3x3 patterns, one hand typically was used exclusively. Interestingly, there did not appear to be a consistent relationship between handedness and the hand employed in block placement. Moreover, there was no consistency across designs for preferred starting position of the first block.

These data indicated that the relationship between handedness and visuo-constructive performance was complex and did not exclusively interact with hand preference for executive-motor performance. Measures of grip strength, writing speed and fine motor coordination support this conclusion. To establish reliability for the assessment of pathological visuo-constructive performance, it is necessary to further explore the nature of this complex relationship with extensive sampling of a normal population.

283.11 EFFECTS OF BRAIN LESIONS ON CUED VISUAL BEHAVIOR IN MAN. David Lee Robinson, Steven E. Petersen*, and Jon Currie*, Laboratory of Sensorimotor Research and Section on Neuro-ophthalmology, National Eye Institute, Bethesda, Maryland 20205.

Reaction times to the onset of a visual target can be influenced by the appearance of prior visual stimuli. In normal control subjects, a light (cue) which correctly predicts the location of a subsequent target will be associated with faster reaction times than cues which incorrectly predict location. Also, weak illumination of the whole visual field (diffuse cue) is associated with slower reaction times. It is hypothesized that valid cues draw attention to the target location and speed reaction times; incorrect or diffuse cues move attention from the correct location and lead to slow reaction times.

Patients with many types of brain pathology have slower reaction times than normal control humans, even when age is not a factor. For patients with specific brain damage we have observed that:

1) Humans with lesions of parietal cortex show significantly slowed reaction times for targets in the contralateral visual field following diffuse and incorrect cues and even for targets in the ipsilateral field following diffuse cues.

2) Humans with damage to the frontal lobes do not show this same pattern of responses. These individuals are only slowed with diffuse cues prior to targets appearing in their affected visual fields.

3) People diagnosed as schizophrenics show slowed reaction times to diffusely cued targets that are in the right visual field.

4) Individuals with Alzheimer's disease generally show such a dramatic overall slowing that the effects of the cue are lost.

5) Humans with occipital lesions which cause a hemianopsia show no influence of the cue when it is presented to their "blind" field; their reaction times are identical to those on trials in which no cue is actually presented, suggesting that the attentional system is dependent on visual data arising from the occipital cortex.

Tests that measure specific capacities can help to understand how different brain areas deal with attentional situations and could also lead to diagnostic markers of brain pathology.

284.1 ONGOING CHANGES IN THE DENDRITIC ARBORS OF INDIVIDUAL SUPERIOR CERVICAL GANGLION NEURONS VISUALIZED IN LIVING MICE. R. D.Hadley and D. Purves. Dept. of Anatomy and Neurobiology, Washington Univ. Medical School, 660 S. Euclid, St. Louis, MO 63110.

A major obstacle to understanding mechanisms of long-term change in the vertebrate nervous system has been the inability to observe the same cell at different times during the life of an animal. We report here a means of making such observations in a relatively simple part of the nervous system. Using this technique we have asked whether the morphologies of dendrites are stable or continuously changing in the superior cervical ganglion of the adult mouse.

The dendritic morphology of single superior cervical ganglion neurons was determined at intervals of up to 3 months in vivo. The cell bodies of neurons at the surface of the ganglion were visualized by asymmetric illumination contrast, and their positions recorded photographically. Neurons were recognized as individuals by their unique shapes, neighbor relationships, and positions with respect to blood vessels. One neuron in each ganglion was arbitrarily chosen and injected with the dye 5(6)-carboxyfluorescein; the dye remains in the neuron for only a few hours. The morphology of the dendritic arbor was recorded with epifluorescence optics and low levels of illumination using a sensitive video camera. After an interval of 3-90 days, the same neuron was identified from the surface photographs and examined a second time by reinjecting it with the fluorescent dye. Approximately 60% of the neurons survived the initial experiment. Comparison of the morphology of the same cell over time showed that the dendrites of these neurons gradually change; little difference was observed at intervals of 1 week, whereas at longer intervals there were progressively more changes of dendritic geometry. The changes were greatest at intervals of 2-3 months. Alterations involved extension of some branches, the retraction or loss of others, and the de novo formation of still other branches.

The progressive nature of these dendritic changes argues that the results we describe are not due to injury; it is unlikely that damage at the time of the initial experiment could continue to stimulate changes many weeks later. Rather, our observations suggest that extension and retraction of dendrites is a normal occurrence in mouse superior cervical ganglion. Since the majority of synaptic contacts occur on the dendrites of these cells, it seems likely that there are corresponding changes in the terminal arborizations of the presynaptic neurons.

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284.2 ALTERATIONS IN HYPOTHALAMIC PEPTIDERGIC NEURONS AND ANGIOARCHITECTURE FOLLOWING RETROCHIASMATIC KNIFE CUTS IN THE RAT. C.P. Phelps and S. Saporta. Dept. of Anatomy, Coll. of Med., U. So. Fla, Tampa, FL 33612

Retrochiasmatic knife cuts made in the frontal plane (FC) have a time dynamic regarding their effect on luteinizing hormone (LH) release function. In brains stained for LH-releasing hormone (LH-RH) using H-15 anti-LH-RH (I. Lengvari) 3 mos after FC, axons containing LH-RH immunoreactivity (ir) are present within and posterior to the FC glial scar in rats that show recovery of LH release after steroid priming. Here we report on the reorganization of peptide-containing neurons (PCN) and angioarchitecture of the mediobasal hypothalamus (MBH) during the first month after FC. PCN were studied using both H-15 anti-LH-RH and Gomori's aldehyde fuchsin (GAF) stain for peptides. Female rats received a right hemi-FC extending 1.5mm laterally and were then processed (Peroxidase anti-peroxidase) for study of LH-RHir 14d later. Immediately posterior to the hemi-FC the anterior median eminence was depleted of LH-RHir terminals and there was the expected accumulation of the peptide rostral to the cut. However, within the FC scar there were also novel LH-RHir positive axons extending dorsally and posteriorly across the cellular wound matrix. The appearance of $GAF^+$ axons in scar tissue 14-30d after FC was a mixture of tangled, beaded structures that appeared to have taken circuitous routes towards or parallel with blood vessels (bv) before forming presumptive terminals on small bv and capillaries. Similar alignments of LH-RHir axons and bv in scar tissue were also seen several months after FC. Studies of angioarchitecture after FC in the retrochiasmatic area (RCA) revealed a transition from a blood filled wound to a well developed microvascular bed in FC scar tissue. Rats were injected with India ink and 4% gelatin in saline through the left ventricle under anesthesia and the brain fixed by immersion in buffered formalin. During the first hours after FC there was poor filling of bv along the lesion border with some leakage of ink into the blood-filled wound. In rats killed 14-30d after FC there was evidence of extensive development of a capillary network and larger (30-40$\mu$) diameter vessels growing in the FC scar tissue. At 30d post-FC the ultrastructure of capillaries within the scar tissue was characterized by a continuous endothelium containing pinocytotic vesicles without fenestrations. In conclusion, FC of neural and vascular connections of the MBH produces a chronology of reorganization in peptidergic neurons and bv during a postoperative period of low pituitary LH release function. The extent of this re-organization was associated in a graded proportion with the degree of eventual recovery of LH release function in individual rats after knife cut lesions. (Supported by the Whitehall Foundation)

284.3 INJURED ADULT NADPH-DIAPHORASE NEURONS SPROUT FIBERS INTO FETAL FRONTAL CORTEX TRANSPLANTS. F.R. Sharp, M.F. Gonzalez, D.M. Ferriero*, and S.M. Sagar, Dept. Neurology, Univ. Calif. SF and VA Med Center, SF, CA 94121.

Data support the existence of transplant-host brain and host-transplanted brain connections particularly in the newborn mammal. Björklund and colleagues have shown that fetal aminergic transplants including dopamine and noradrenergic cells send fibers for some distances into adult host brains and may reverse behavioral deficits due to chemical deficiences in the adult host brain. There is little data to suggest that adult host brain might send connections into fetal transplants with the exception of growth of adult cholinergic fibers into fetal hippocampal transplants ( Kromer et al., Brain Res., 153-200, 1980, 1981). We now provide evidence for growth of adult host brain cell fibers into surviving fetal frontal cortex transplants.

Adult albino rats were anesthetized and the right motor cortex removed (Sharp and Gonzalez,Neurology,34:1305-1311,1984). After surviving one week, hosts were reanesthetized and frontal cortex from 17-19 day old embryos placed in the host cavity. Cavities were sealed and host rats allowed to survive one to three months. They were reanesthetized and perfused with phosphate buffered 3% paraformaldehyde. Brains were removed and 50$\mu$m thick sections cut on a vibratome and reacted for NADPH-diaphorase (Scherer-Singler et al., J. Neurosci. Meth., 9:229-234, 1983). The results revealed that many blue stained cells and their processes were always seen in normal adult neocortex. Two types of stained transplants were found. Some had NADPH-diaphorase stained cells and fibers throughout the transplant which was comparable to host neocortex. In others there were no stained cells in the transplant, but there were a few fibers at the transplant-host brain interface which could be traced from the host brain into the transplant for as much as a millimeter in some cases. NADPH-diaphorase stained sections of 17-19 day old fetal brain revealed few fibers and rare cells in the developing neocortex.

It has been suggested that NADPH-diaphorase may be a selective histochemical marker for neocortical and striatal neurons containing somatostatin and Neuropeptide Y (Vincent et al., J. Comp. Neurol., 217:252-263, 1983). This data and our results suggest that injured adult host brain neurons sprouted fibers into the fetal transplant, and that these NADPH-diaphorase staining fibers may be peptidergic.

284.4 Morphological Characteristics of Fetal Cortex Implants in Adult Rats Following Cortical Ablation. R. Labbe, E.J. Mufson, and D.G. Stein, (SPON: T.A. Schoenfeld) Clark University, Worcester, MA and Harvard Med. Sch. Boston, MA.

Previously, we found that implants of fetal cortical tissue partially correct the cognitive deficits resulting from damage to frontal cortex in adult rats and that these implants form neural connections with the host brain (Labbe et al. 1983). The present study is a more extensive analysis of the morphological characteristics of fetal frontal cortex implants.

Seven days after aspiration of the medial frontal cortex in 100 day old male C-D Sprague-Dawley rats, animals received implants of frontal cortex tissue (approximately 6 $mm^3$) from day 19 C-D rat fetuses. Approximately 100 days after implantation, the animals were perfused, the transplant tissue was reacted for the enzymes acetylcholinestrase (AChE), choline acetyltransferase (ChAT), cytochrome oxidase, and stained for myelinated fibers and with thionin.

Transplants either formed continuous bridges connecting injured hemispheres or formed separate grafts, each adhering to the host cortex. Thionin-stained sections revealed little internal order or laminar arrangement of neurons characteristic of intact frontal cortex.

Tissue processed for AChE histochemistry (Hedreen et al. 1985) revealed AChE positive fibers and cell bodies throughout the implants. The distribution of fibers was nonlaminar and some AChE positive fibers appeared to cross the host-transplant border. Other enzyme positive fibers, were seen coursing within neural bridges interconnecting portions of the transplant. AChE processed tissue counterstained with thionin showed non-AChE positive neurons intermixed with enzyme containing cell bodies. Sections processed with butrylthiocholine as a substrate revealed an extensive esterase positive vascular network. ChAT immunohistochemistry showed only a few ChAT positive fibers and perikarya in the transplant.

Implant tissue stained for the mitochondrial enzyme cytochrome oxidase revealed regional variations in activity levels within the implants. Sections stained for myelinated fibers showed long interconnecting tracts along the exterior surface as well as a reticularlike network of fibers within the transplant.

In conclusion, although frontal cortex implants fail to exhibit a distinct laminar architectonic profile, they share many of the intrinsic morphological characteristics of intact frontal cortex.

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284.5 LAMININ STIMULATES NEURITE OUTGROWTH FROM RAT RETINAL EXPLANTS. T. S. Ford-Holevinski*, J. M. Hopkins, P. J. McCoy* and B. W. Agranoff (SPON: G. J. Siegel). Mental Health Research Institute and Department of Pathology, University of Michigan, Ann Arbor, MI 48109.

Recent experiments have suggested that the failure of the mammalian CNS to regenerate after injury is due to a deficient or hostile extraneuronal environment, since CNS neurons will grow axons through properly grafted PNS tissue. We here compare the teleost visual system, a model of successful CNS regeneration, and the adult rat visual system, in which regeneration fails, to elucidate the relative significance of intrinsic and extrinsic factors related to nerve regeneration. We previously demonstrated that retinal explants from goldfish with prior-lesioned optic nerves extend neurites onto a surface coated with the basal lamina component laminin at a much higher rate than is observed with other substrata (Hopkins et al., J. Neurosci., in press). In vivo, there was a generous laminin investment surrounding the goldfish optic nerve fascicles, presumably due to invaginations of the pial membrane. Furthermore, we observed a dramatic increase in laminin immunoreactivity within the fascicles after these nerves were lesioned or their retinas removed. Since it is also known that laminin is prominent in mammalian peripheral nerve, these findings were taken to suggest that laminin plays an important role in the ability of a nerve to regenerate following injury. We have now examined the optic nerve of the rat for the presence of laminin before and after axotomy. Unlike the goldfish, laminin immunoreactivity was restricted to the nerve sheath and vascular endothelium, and axotomy did not result in incremental laminin staining within the nerve. Explants of prior-lesioned retinas from adult rats were found to extend neurites in vitro, and the proportion of explants exhibiting neuritic growth was significantly enhanced by the presence of laminin in the substratum. Neurite outgrowth from explants of unlesioned rat retinas was minimal. Thus, retinal ganglion cells of both the goldfish and the adult rat possess the intrinsic ability to regrow their axons in primary tissue culture following a conditioning lesion if provided with a suitable substratum, optimally laminin. We suggest that the failure of the rat visual system to regenerate after axotomy in vivo may be due in part to the inability of supporting cells of the mammalian optic nerve to provide a laminin-containing matrix in response to injury. (Supported by NEI Grant EY 05947, NIH Training Grant MH 15794 and NIH Training Grant 5 T32 MH 14279).

284.6 GLIAL FIBERS IN ADULT CANARY BRAIN ARE THOUGHT TO ACT AS PATHWAYS FOR PUTATIVE MIGRATING NEUROBLASTS. F. Nottebohm, D.R. Buskirk, G.D. Burd and B. O'Loughlin*. Rockefeller University, New York, N.Y. 10021.

Monoclonal antibodies were prepared against the crude plasma membrane fraction of forebrain homogenate from adult male canaries and were screened in cryostat sections of adult canary brain using immunofluorescence. One antibody, an IgM designated III-40C-E3, stained perivascular astrocytes, fibrous astrocytes, brain surface astrocytes and tanycytes as well as Bergman glial fibers in the cerebellum and long processes that extend from ventricular zone cells that line the lateral ventricles of the forebrain.

The processes extending from the lateral ventricle are of special interest to us because we hypothesize that they may serve as pathways for young migrating neurons that are generated in the ventricular zone of adult canaries (Goldman and Nottebohm, PNAS 80:2390, 1983). To test our hypothesis, we pretreated adult male and female canaries with 3H-thymidine (2.5 μCi per g body wt, twice daily for 14 days) to label newly generated neurons and processed the tissues for immunocytochemistry using the unlabeled peroxidase method followed by autoradiography. Labeled neurons were observed in the forebrain 30 days after the first 3H-thymidine injection, and at this time had no particular association with processes immunoreactive with III-40C-E3. At short survival times (15 days after the first 3H-thymidine injection) small, elongated cells, located in the forebrain close to the ventricular zone, were labeled with 3H-thymidine and tightly apposed to long processes that were immunoreactive with III-40C-E3. These processes were extensions of cells that lined the lateral ventricle.

III-40C-E3 shows immunoreactivity in vitro with the cytoskeleton of vimentin-positive tissue culture cells. The immunocytochemical staining of astrocytes and Bergman glia fibers suggests that this antibody may also recognize glial fibrillary acidic protein.

We believe that at least some of the small elongated cells described above are generated in the ventricular zone and migrate along glial processes toward their final destination in the forebrain. This is similar to what is thought to occur in parts of the developing brain and is the first report of migration of putative neuroblasts along glial fibers in the adult brain.

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284.7 SEPARATE CLASSES OF INTERNEURONS IN A SONG CONTROL NUCLEUS: THOSE SHOWING GABA IMMUNOREACTIVITY AND THOSE BORN IN ADULTHOOD. G.D. Burd, J.A. Paton, and Fernando Nottebohm. Rockefeller University, New York, NY 10021.

Neurons continue to be generated and incorporated into the forebrain of canaries during adulthood. (Goldman and Nottebohm, PNAS 80:2390, 1983). This addition of neurons is especially prominent in hyperstriatum ventralis, pars caudalis (HVc), a forebrain nucleus that is an essential part of the motor pathway controlling vocalization in song birds. New neurons located in this nucleus are predominately local interneurons (Paton, et al., J.Neurosci., in press). In this abstract, we report that cells containing GABA immunoreactivity have been found throughout the forebrain, including HVc, and that within HVc, these cells are also local interneurons, but are not new neurons.

The first set of experiments tested the hypothesis that the newly generated neurons contain GABA. In these experiments, adult canaries were pretreated with $^{3}$H-thymidine (2.5 μCi per g body wt, twice daily for 14 days). After 30-45 days, these canaries were perfused with 1% paraformaldehyde and 1.25% glutaraldehyde and tissue sections from the forebrain were processed for immunocytochemistry using GABA-BSA (ImmunoNuclear Corp.) or GABA-KLH (Hoskins, et al., Soc. Neurosci. Abstr., 1984) antisera and the unlabeled antibody peroxidase method; this was followed by autoradiography. While new neurons and neurons immunoreactive for GABA are numerous and widely distributed throughout the forebrain, only rare neurons were doubly labeled with $^{3}$H-thymidine and GABA immunoreactivity. No doubly labeled neurons were observed in HVc.

In a second set of experiments, we examined the potential axonal projections of HVc neurons that contain GABA immunoreactivity. In these experiments, projection neurons in HVc were labeled by retrograde transport of rhodamine-coupled latex beads (Katz, et al., Nature 310:498, 1984). The beads were injected into the two nuclei known to receive axonal projections from HVc neurons - robust nucleus of the archistriatum (RA) and area X. After 2-3 days, tissue sections from the forebrain of these canaries were processed for immunocytochemistry, as described above. No cells with GABA immunoreactivity were labeled with fluorescent beads. Thus, the GABA immunoreactive neurons in HVc are not projection neurons, and therefore are local interneurons.

We conclude that HVc contains two separate classes of local interneurons, those that contain GABA and those that are added during adulthood. This implies that the pool of new neurons is only a subset of the interneurons located in HVc.

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284.8 ALTERATIONS IN NODE OF RANVIER STRUCTURE THAT ACCOMPANY ELECTROPHYSIOLOGICAL STIMULATION ARE EFFECTED BY PHARMACOLOGIC AGENTS. Charles C. Wurtz and Mark H. Ellisman. Lab. for Neurocytology, Dept. of Neurosciences, UCSD, La Jolla, Ca. 92093.

We recently reported on alterations in the ultrastructure of peripheral nodes of Ranvier that are associated with repetitive action potential propagation (Wurtz & Ellisman, Nsci. Abs. 10:865, 1984). Morphologic changes included the appearance of extracellular intramyelinic vacuoles and an alteration in the axonal matrix within the nodal region. These changes were easily induced by activation of frog dorsal roots in situ at 50 Hz for 15 min. This stimulation also results in slowed conduction velocity (CV) of the compound action potential (CAP) and a reduced amplitude indicative of fiber drop-out. Fibers recover from the stimulation associated paranodal disruption and axonal matrix alteration if incubated in Ringers for 30 minutes after stimulation is halted. To better understand the basis for the observed alteration and recovery, a series of experiments using pharmacologic agents to manipulate metabolic and ionic conditions were performed during the stimulation and recovery phases.

The observations that appear most interesting at present are from two different experiments using drugs that disturb the movement of ions across membranes. In one series, 4-aminopyridine (4-AP) was employed to block $K^+$ channels during stimulation, while in another series ouabain was included during the recovery to inhibit the $Na^+$+$K^+$ATPase. To block or reduce $K^+$ conductance 250μM 4-AP was included in the bathing media superfusing the excited portion of the nerve throughout 15 min. of stimulation at either 50 or 100 Hz. Morphologic data indicates that the paranodal vacuolization previously shown to occur at these activation frequencies is reduced in magnitude. This is accompanied by a slowing of the CAP and a broadening of its waveform, but not by a significant decrease in amplitude, suggesting that fiber drop-out is not pronounced under these conditions.

To investigate the dependence of recovery on active transport we added 1.5mM ouabain to the superfusate during the recovery phase. Morphologic recovery was substantially inhibited in ouabain treated fibers, which exhibited paranodal vacuolization even if fixed after 45 min. of recovery. The CAP of the ouabain treated preparations displayed both a substantial reduction in CV from the pre-stimulation state, and a smaller reduction from the CV measured at the end of the 15 min. of stimulation at 50 Hz. In other words recovery in ouabain further depresses CV. The amplitude of the CAP, however, recovered to a value 15-20% greater than the pre-stimulation value. These results may be interpreted to implicate the involvement of $K^+$ movements within the paranodal-nodal complex in modulating structural effects and, subsequently, characteristics of action potential propagation.

284.9 CALCIUM-DEPENDENT LONG-TERM ENHANCEMENT OF SYNAPTIC TRANSMISSION WITHOUT ACTION POTENTIALS IN AXONAL TERMINALS AT THE CRAYFISH NEUROMUSCULAR JUNCTION. J.M. Wojtowicz and H.L. Atwood, Dept. of Physiology, University of Toronto, Toronto, Ont. M5S 1A8.

The neuromuscular junction of the crayfish (Procambarus clarkii) opener muscle was studied in vitro using electrophysiological methods. One microelectrode was placed in the terminal region of the excitatory axon and another in the underlying muscle fiber. Excitatory postsynaptic potentials (EPSPs) were evoked in muscle fibers at a frequency of 5 Hz by remote stimulation of the axon. Tetanic stimulation at 20 Hz for 10 minutes resulted in a large, gradual enhancement (>15 fold) of the EPSPs which recovered to a level 50-100% above the baseline 30 min after the tetanus. The phenomenon is known as long-term facilitation (LTF). LTF can be accounted for by accumulation and subsequent decay of Na and Ca in the terminals (Wojtowicz and Atwood 1983, Neurosci. Abst Vol. 9 p. 169). However, a significant part of the enhancement remains for periods in excess of 30 minutes following the tetanus and its ionic mechanism has not been studied rigorously. The purpose of the present study was to investigate its mechanism using a new technique of local stimulation. Presynaptic action potentials were abolished by exposure to 30 nM TTX. Passive, depolarizing pulses 3-5 msec in duration were produced by intra-axonal current injections through the presynaptic electrode. The amplitude of the pulses was adjusted to obtain the same average amplitude (0.3-0.6mV) of EPSPs as measured during evoked activity prior to the TTX treatment. The resulting release of transmitter was synchronous, quantal in nature and mimicked the EPSPs. This mode of stimulation was localized to a few terminal branches and the possiblity of activating processes of any other axons was eliminated. In 15 preparations LTF was produced by such local stimulation at 20 Hz for 10 min. The component of LTF which is normally caused by influx of Na into the axon was absent in these TTX treated preparations. However, a long-term enhancement (50-100%) of transmitter release was present at 40 min following the tetanus. This effect was fully accounted for by the increase of quantal content of the EPSPs, but not of quantal size. Binomial analysis (Wojtowicz and Atwood 1984, Neurosci. Abst Vol. 10 p. 666) indicated an increase in the number of active, synaptic release sites. Furthermore, substitution of Ca with equimolar concentration of Mg (in the presence of 1mM EGTA) or with 6mM Mn for the duration of the tetanus blocked transmitter release during the tetanus as well as the long-term enchancement. We conclude that the long-lasting enhancement of synaptic transmission is a presynaptic effect confined to the terminal branches of the excitatory axon. The presence of calcium is necessary for the establishment of this phenomenon. Supported by MRC of Canada.

284.10 SYNDACTYLY RESULTS IN THE EMERGENCE OF DOUBLE-DIGIT RECEPTIVE FIELDS IN SOMATOSENSORY CORTEX IN ADULT OWL MONKEYS. T.T. Allard, S.A. Clark*, W.M. Jenkins, and M.M. Merzenich. Coleman Laboratory, Departments of Otolaryngology & Physiology, UCSF, San Francisco, CA 94143; and Department of Plastic & Reconstructive Surgery, Stanford University, Stanford, CA 94305.

Surgical syndactyly or cutaneous fusion of two adjacent digits in adult owl monkeys induced central reorganization in the representation of the two digits as observed in Area 3b of somatosensory cortex. Both the dorsal and glabrous surfaces of digits 3 and 4 were surgically joined from the proximal web space to the distal tips in 3 monkey hands. A single detailed cortical map of digits 3 and 4 was obtained in these three cases at 3, 5½, or 7½ months after surgical syndactyly. During the mapping procedure, receptive fields were defined for clusters of units in a series of microelectrode penetrations in a fine grid (mean interpenetration distances ranged from 112 to 172 microns). In all three cases, penetrations in about 30% of the cortical sector representing digits 3 and 4 had receptive fields that overlapped both fingers: Case 1 had 46 double-digit receptive fields; Case 2 had 49 double-digit fields; Case 3 had 32 double-digit fields. In contrast, double-digit fields were recorded infrequently in normal maps, and were observed in only a few instances at the D2-D3 and D4-D5 borders in these experimental monkeys. The double-digit fields of the syndactyly were equally distributed across the proximal, middle and distal phalanges. As in a normal map, glabrous fields outnumbered dorsal fields by approximately 3 to 1.

Immediately after two of these maps were completed, the syndactyly was released surgically and additional penetrations made at or near double-digit recording sites. In one case, 20 of 21 penetrations showed response to stimulation of each separate digit. Fifteen of these sites had cutaneous fields on both fingers. In the second case, 15 of 17 post-release penetrations revealed double-digit fields. These release data support the view that the double-digit representation reflects a central reorganization rather than cross-digit peripheral nerve regeneration in the hand. In 7 penetrations, physically discontinuous receptive fields were formed at the distal tips of D3 and D4, again suggesting that central rather than peripheral mechanisms underly the emergence of double-digit fields.

Results of this study are consistent with our operating hypothesis: cortical receptive fields are derived from extensive input repertoires through an input selection process based on the temporal correlations of specific inputs. They also demonstrate that the normal representational discontinuities between digits in Area 3b are established by use through the operation of intrinsic, central somatosensory system processes.

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284.11 PLASTICITY OF SOMATOSENSORY MAPS IN THE ADULT MAMMALIAN SPINAL CORD

P.J. Snow* and P. Wilson* (SPON: J. Pettigrew), Department of Anatomy, University of Queensland, St. Lucia, Brisbane, Australia.

Previous attempts to demonstrate plasticity in the neural connections underlying the somatosensory maps in the dorsal horn have utilized the steep mediolateral gradients in somatotopic organization. There is currently considerable disagreement between workers on whether or not such changes do in fact occur (Devor, M. & Wall, P.D. Nature, 267: 75, 1978; Lisney, S.J.W. Brain Res., 259: 31, 1983; Brown, A.G. et al. J. Physiol., 354: 375, 1984). The demonstration that cutaneous primary afferents have projections to those somatotopically inappropriate regions of the spinal grey which are rostrally and caudally displaced from the appropriate region (Meyers, D.E.R. et al. Neurosci. Letts., 44: 179, 1984) suggested to us that reorganization along the rostrocaudal axis might be more easily demonstrable. Previous work in our laboratory has shown that in both the adult and neonatal cats the toes are precisely represented in a rostrocaudal order from toe 2 to toe 5. The present experiments were aimed at studying the effects of acute (same day) and chronic (30-70 days survival) denervation of toe 3 on dorsal horn somatotopy. Experiments were conducted on 2 cats in which all toes were ligated acutely, on 1 cat in which all toe 3 nerves were ligated chronically and 6 cats in which these nerves were ligated chronically on one side and actuely on the other. Animals were anaesthetized with α-chloralose (70 mg/kg), paralysed with gallamine and artificially respired. Recordings on both sides of the cord were made from a total of 235 antidromically identified spinocervical tract cells and 20 unidentified dorsal horn neurons using pontamine filled microelectrodes. After acute denervation no cutaneous receptive fields were found on toe 3. After chronic denervation cells in the toe 3 area responded to brisk hair movement on areas of skin surrounding the denervated toe. Dissection of the foot showed that no regeneration of the ligated nerves had occurred. We conclude that even though somatotopic organization is present in the neonatal kitten rostrocaudal reorganization does occur in somatotopic maps of adult cats following nerve section.

284.12 REPEATED AFFERENT STIMULATION AND EXERCISE RESTORE MOTOR FUNCTION IN HUMANS WITH CHRONIC SPINAL CORD INJURY.

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The prognosis for humans with spinal cord injury is poor because effective regeneration is prevented by physical barriers such as scarring and glial proliferation (W.J.Freed et al., Science 227: 1544, 1985). On the other hand, neurophysiological evidence indicates a sparing of some spinal pathways even in apparently complete injuries (J.Walker, Science, 216: 203, 1982), and stimulation can alter spinal cord circuitry for prolonged periods of time (Mendell, Physiol. Rev., 64: 260, 1984). We therefore investigated the effects of repeated afferent stimulation, in the form of photostimulation with a low power helium-neon laser (J.Walker, Soc Neurosci Abst 14: 866, 1984) and exercise on neurological function in subjects with chronic traumatic spinal cord injuries ranging from 1-10 years duration.

Twenty nine healthy subjects (21 quadriplegics and 8 paraplegics) received a full day evaluation which included videotaped quantitative neurological assessment, cardiovascular evaluation, cortical somatosensory evoked potentials, and review of medical records including radiological studies. After acceptance, subjects were placed on a daily program that consisted of physical therapy (standing, passive range of motion, resistance exercise, aerobics, gait training) and eight 40 second exposures with a low power laser to skin overlying the peripheral nerves. Participation of each subject ranged from 3 months to 1 year. Videotaped quantitative neurological assessment and electrophysiological studies were repeated every six weeks.

All subjects demonstrated a 10-100% improvement in overall muscle strength, and 26/29 subjects exhibited a return of muscle function below the level of the lesion (range: 2-20 muscles). Eighteen of the 20 quadriplegics who had absent cortical SEP's to median nerve stimulation exhibited a return of the cortical SEP's ($F=12.1$ $df=47$ $p<.001$). Only two of the eight paraplegics exhibited an improvement in cortical SEP's to posterior tibial nerve stimulation. Eighteen untreated subjects showed no change in 6 months of observation.

Improvements in neurological function were accompanied by the return of the ability to ambulate in 10/21 quadriplegics and 8/8 paraplegics. The subjects use walkers or crutches as assistive devices and their range varies from 50 feet to 1.5 miles. The neurological basis of this surprising recovery is under investigation. These results suggest that exposure to such rehabilitation may optimize the results of future experimental interventions such as neural transplantation.

285.1 SOMATOSTATIN HAS AN EXCITATORY EFFECT ON SPINAL NOCICEPTIVE MECHANISMS: BEHAVIORAL AND ELECTROPHYSIOLOGICAL STUDIES. Z. Wiesenfeld-Hallin. Department of Clinical Neurophysiology, Huddinge University Hospital, S-141 86 Huddinge, Sweden.

Somatostatin (SOM) is present in the central terminals of some small diameter primary afferents, presumably nociceptors. Yet the role of SOM in spinal sensory mechanisms is unresolved. On the one hand, it has been reported that intrathecally (IT) applied SOM induces behaviors indicative of unpleasant sensations in conscious animals (Seybold et al, 1982, Peptides 3, 49). On the other hand, physiological studies (Randic and Miletic, 1978, Brain Res. 152, 196; Suzue et al, 1981, Neurosci. Lett. 26, 137; Yanagisawa et al, 1985, Europ. J. Pharmacol. 106, 231) have indicated that SOM has an inhibitory effect on the spinal cord. In this study both behavioral and electrophysiological experiments were carried out in rats using a wide dose range of SOM in order to resolve these inconsistencies.

In the behavioral experiments the rats were implanted with IT catheters and 100 ng - 25 µg SOM (Ferring) was injected onto the lumbar enlargement. The modulation of the hamstring flexion reflex to stimulation of nociceptive afferents by 10 ng - 10 µg SOM IT was studied in decerebrate, spinal, unanaesthetized rats. It has been found that this reflex can be facilitated by conditioning stimulation of C-afferents in peripheral nerve (Wall and Woolf, 1984, J. Physiol. 356, 443) and IT injection of substance P and calcitonin gene related peptide (Woolf and Wiesenfeld-Hallin, in press).

In the behaving animal 1 and 10 µg, but not 100 ng, SOM caused a dose-related scratching response lasting about 20 min directed toward the hind limbs and flanks, thus confirming previous results. The 25 µg dose caused paralysis of the hindlimbs. In the physiological experiments low doses of SOM facilitated the flexion reflex, whereas the highest dose blocked the response.

In conclusion, at low doses SOM had an excitatory effect on spinal nociceptive mechanisms and "inhibition" was a result of blockade due to application of excessively high doses. Furthermore, the doses causing excitatory responses were considerably lower in the physiological preparations than in conscious animals.

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285.2 EFFECTS OF PHYSALAEMIN ON FUNCTIONALLY IDENTIFIED SPINAL DORSAL HORN NEURONES IN THE CAT. M.W. Salter and J.L. Henry, Depts. Physiol. & Anaes. Res., McGill Univ., Montreal, Que., H3G 1Y6.

Physalaemin is an agonist of the SP-P type of tachykinin receptor in peripheral tissues (Iversen,L.L., Br. Med. Bull. 38:277, 1982). Tachykinins endogenous to the mammalian dorsal horn include substance P, which has been implicated as a possible mediator of excitatory synaptic transmission in nociceptive pathways (Henry,J.L., Br. Res. 114:439, 1976). To further investigate the role of tachykinins in spinal sensory mechanisms an electrophysiological study was done on the effects of iontophoretic application of physalaemin on dorsal horn neurones.

Experiments were done on 26 cats: 12 were anaesthetized with alpha-chloralose (60 mg/kg, i.v.), 11 were anaesthetized with Na-pentobarbital (40 mg/kg, i.p.) and 3 were decerebrated at the intercollicular level. All cats were acutely spinalized at L1. Extracellular, single unit spikes were recorded in segments L5-L6 with one barrel of seven-barrelled micropipettes. Physalaemin (1mM in 165 mM NaCl,pH 5.5), substance P (0.8 mM in 165 mM NaCl, pH 5.5) and NaCl (165 mM,pH 5.5) were each contained in separate barrels for iontophoresis. Units were classified by responses to peripheral stimuli as wide dynamic range (WDR), non-nociceptive (NN), nociceptive specific (NS) or proprioceptive (PR).

Physalaemin (40-150 nA; 45-150 sec) excited 46 of 55 units. Excitation began 30-120 sec after the start of ejection, increased to a maximum about 1 min after the end of ejection and disappeared gradually over 2-5 min. Effects on the functional groups of neurones were:

| | WDR | NN | NS | PR |
|---|---|---|---|---|
| Excitation | 35 | 5 | 2 | 4 |
| No effect | 5 | 4 | 0 | 0 |

Twenty-four units were tested both with physalaemin and with substance P. Thirteen units were excited by both peptides, 7 were excited by physalaemin but not by substance P, one was excited by substance P but not by physalaemin and 3 were unaffected by both. Of non-nociceptive units 3 of 4 were excited by physalaemin while none was affected by substance P.

These results indicate that physalaemin not only excites nociceptive units, as might expected from earlier studies with substance P, but also excites non-nociceptive units. Thus, tachykinin receptors appear to be present on both nociceptive and non-nociceptive neurones. However, in view of the observation that non-nociceptive neurones are unaffected by substance P, tachykinin receptors on non-nociceptive neurones are probably different from those on nociceptive neurones.

(Supported by the Medical Research Council of Canada)

285.3 BRADYKININ-ACTIVATED TRANSMEMBRANE SIGNALS ARE COUPLED VIA $N_i$ OR $N_o$ TO INOSITOL-1,4,5-TRISPHOSPHATE, A SECOND MESSENGER IN NG108-15 NEUROBLASTOMA X GLIOMA CELLS. Haruhiro Higashida*, Werner Klee, David Brown, and Marshall Nirenberg. NIH, Bethesda, MD., 20205.

The addition of bradykinin (BK) to NG108-15 cells results in a transient outward transmembrane current (hyperpolarization) followed by a prolonged inward current (depolarization). Concomittantly, BK also increases the rate of hydrolysis of phosphatidyl-inositol-4,5-bisphosphate (PI-$P_2$) yielding inositol-1,4,5-trisphosphate ($IP_3$) and diacylglycerol (DAG), presumably by activation of phosphatidyl-4,5-bisphosphate phosphodiesterase (PI-$P_2$ PDE), and increases the rate of secretion of acetylcholine from NG108-15 cells. (Yano et al. J. Biol. Chem. 259, 10201 (1984), and FEBS Letters 181, 403 (1985)). Injection of $IP_3$ or $Ca^{2+}$ into the cytoplasm of NG108-15 cells results in cell hyperpolarization followed by depolarization, similar to the effects elicited by extracellular BK. $IP_3$ stimulates the release of $Ca^{2+}$ ions from internal storage sites into the cytoplasm in NG108-15 cells (Ohsugi et al. personal commun.). Cell membrane permeability increases during the hyperpolarization phase, and the hyperpolarization reverses at approximately -75 to -80 mV. These results suggest that elevation of cytoplasmic $Ca^{2+}$ ions activate $Ca^{2+}$-dependent $K^+$ channels resulting in cell hyperpolarization. BK also stimulates uptake of extracellular $^{45}Ca^{2+}$ by cells. BK-dependent cell depolarization decreases to 20% of the control value when cells are incubated in medium without $Ca^{2+}$ or with $Co^{2+}$. Membrane resistance increases during the depolarizing phase of the cell response to BK, which suggests that the rate of $K^+$ efflux via M channels is reduced. In contrast, elevation of intracellular $IP_3$ decreases membrane resistance, but has no effect on M channel activity.

The mean amplitudes of the hyperpolarization and depolarization responses of NG108-15 cells to BK decrease approximately 50% (maximum inhibition) after cells are incubated with 200 ng pertussis toxin (PT)/ml for 15-20 hr. BK stimulates low $K_m$ GTPase activity in NG108-15 membrane preparations, but not in membranes prepared from NG108-15 cells treated with 10 ng of PT/ml for 18 hr. Incubation of NG108-15 membranes from PT treated cells with an approximately equimolar mixture of purified $N_i$ and $N_o$ (>95% pure) from bovine brain restores BK-dependent GTPase activity. BK also inhibits basal adenylate cyclase activity in NG108-15 homogenates. These results show that [BK·Receptor] complexes interact with $N_i$ and/or $N_o$ and suggest that $N_i$ and/or $N_o$ may couple BK activation of receptors with activation of low $K_m$ GTPase and PI-$P_2$ PDE and with inhibition of adenylate cyclase.

285.4 NEUROPEPTIDE Y REDUCES RAT HIPPOCAMPAL CA1 POPULATION SPIKE AND MONOSYNAPTIC EPSP BY PROBABLE PRESYNAPTIC ACTION. William F. Colmers, Ken Lukowiak* and Quentin J. Pittman. Department of Medical Physiology, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

Neuropeptide Y (NPY) has been localized in cell bodies and fibers of the hippocampus and dentate gyrus. Because NPY has potent effects on the physiology of synaptic transmission in peripheral contractile tissues, we wished to examine its effects on synaptic transmission in the hippocampus.

Hippocampal slices from 75-100 g male Sprague-Dawley rats were incubated, submerged, at 32°C, and perfused at 2-3 ml/min with oxygenated artificial CSF. A bipolar stimulating electrode was placed on stratum radiatum to elicit orthodromic, and on alveus to elicit antidromic, population spikes (PS's), which were recorded with an extracellular microelectrode in the CA1 cell body layer. PS's were averaged and stored with a digital oscilloscope. Intracellular recordings were made with $K^+$ acetate electrodes.

Application of $10^{-6}$M NPY for 4 minutes caused a reduction of the PS to 14.01 ± 4.17% of control amplitude. Threshold for effect was $10^{-8}$M; $10^{-9}$ to $10^{-10}$M had no significant effects. Effects of NPY were completely reversible; $10^{-6}$M required 1-1.5 hours washout. There appeared to be no difference between NPY's effects on single pulse or paired pulse (ISI=30 msec) facilitated PS's. $10^{-6}$M NPY had no effect on PS's evoked by antidromic stimulation from alveus.

In another series of experiments, CA1 pyramidal neurons were impaled concurrently with the extracellular field potential recording. By varying the stimulus voltage, stimulus-response relationships could be constructed for both the PS and EPSP amplitudes. Application of $10^{-6}$M NPY to healthy (20-30mV action potential overshoot, > 30MΩ input resistance) CA1 cells reduced the amplitude of the evoked EPSP to 63.78 ± 6.55% of control when measured at the midpoint of the stimulus-response curve, and to 71.29 ± 6.77% of control at the maximum of the curve (n=8, $P < 0.05$). The resting potential, the number and amplitude of action potentials evoked by a current pulse of constant amplitude, and input resistance of the cells were not affected. NPY had a greater effect on the PS amplitude than on the EPSP. The lack of apparent effect of NPY on the passive membrane properties and action potentials of these cells, along with its action on afferent inputs to these cells, suggests strongly a presynaptic action of NPY.

Supported by MRC of Canada. WFC is an AHFMR Fellow; QJP is an AHFMR Scholar and MRC Scientist.

285.5 EFFECT OF NEUROHYPOPHYSEAL HORMONES AND SOME OF THEIR FRAGMENTS ON SYNAPTICALLY EVOKED FIELD POTENTIAL IN LATERAL SEPTUM OF RATS. M.Joëls* and I.J.A.Urban* (SPON: European Neuroscience Association). Rudolf Magnus Institute for Pharmacology, State University of Utrecht, Vondellaan 6, 3521 GD Utrecht, The Netherlands.

Recent experiments showed that iontophoretically applied arginine$^8$ vasopressin (AVP) excites part (30%) of the neurons in the lateral septum (LS).Moreover, in the majority of the LS cells AVP induces a long-term increase in the cell responses to excitatory amino acids. Thus, in ca. 65% of the LSC cells responses to exogenously applied excitatory amino acids were markedly increased by AVP and remained elevated for at least 15 minutes after the peptide administration. The enhanced responsiveness to exogenously applied amino acids was accompanied by an increase in the LS cell responses to endogenously released amino acids: Single unit and field responses evoked in the LS by stimulation of the fimbria and fornix, which constitute an excitatory amino acid mediated projection to the LS, were increased after local application of AVP. In the present study we addressed two questions: (i) Is this facilitatory effect on amino acid transmission specific for AVP or can substances structurally related to AVP, e.g. oxytocin (OXT), desglycinamide AVP (DGAVP) or 1-deamino-D-AVP (dDAVP) exert similar actions; (ii) Is it possible that the facilitatory action evoked by AVP is the result of the action of one of the AVP-metabolites as was postulated with regard to the behavioral effects of AVP. To answer these questions we examined the effect of AVP, OXT and related fragments on the amplitude of the synaptically evoked field potential (FP) negativity, comprising excitatory postsynaptic potentials induced in LS cells by fimbria-fornix stimulation. The compounds were dissolved in artificial cerebrospinal fluid and perifused (0.2ml/min) on the septal surface at various concentrations. It appeared that AVP increased the FP negativity already at a concentration of $10^{-10}$M. Like AVP, $10^{-8}$M OXT enhanced the FP amplitude; with $10^{-10}$M OXT the effect was no longer significant. DGAVP also induced a significant increase in the FP negative wave but here at least $10^3$-fold higher concentrations than of AVP were needed. Increases induced by the antidiuretic agonist dDAVP ($10^{-7}$M) were not significant. A significant increase of the FP negativity was evoked by the AVP-metabolites [pGlu$^4$,Cyt$^6$]AVP(4-9) and [pGlu$^4$,Cyt$^6$]AVP(4-8) at $10^{-6}$M concentrations, whereas only a weak increase was observed with $10^{-7}$M.A small increase was observed with $10^{-6}$M of [Cyt$^6$]AVP(5-9). OXT-metabolites tested at $10^{-7}$M doses did not affect the amplitude of the FP. These results demonstrate that the facilitatory effect of OXT and some of the neurohypophyseal hormone-fragments on the excitatory amino acid mediated synaptic input to the LS is similar to the AVP-effect. Yet, the considerably lower effective dose of AVP in comparison to the dose of the other compounds indicates that (i) the facilitatory effect is to a great extent specific for AVP and (ii) the effect observed with AVP is not likely to be the result of the action of one of its metabolites.

285.6 CHOLECYSTOKININ ACTS AS AN EXCITATORY NEUROMODULATOR IN RAT AMYGDALA. R.B. Innis and G.K. Aghajanian. Dept. of Psychiatry, Yale University School of Medicine, New Haven, Connecticut 06508.

Cholecystokinin is a neuropeptide which is widely distributed in the CNS of many species. CCK has fulfilled most criteria for neurotransmitter status, including excitatory actions in many areas of rat CNS. The amygdala is enriched in fibers which stain for CCK-like immunoactivity and in binding sites for CCK radioligands. Therefore, we are studying the electrophysiologic actions of CCK iontophoretically applied in rat amygdala.

We have used the technique of single unit recording with a 5-barrel micropipette in transected rats (cerveaux isole preparation) with local anesthetic to pressure points. The naturally occurring sulfated form of cholecystokinin octapeptide (0.25 mg/ml CCK-8 in normal saline, pH 7) was ejected as a negative current. The position of the electrode was determined by passing a dye spot at the end of each experiment, and to date, we have studied cells in the nuclei centralis, corticalis, and basalis of the amygdala. Most amygdaloid neurons were not spontaneously active and were driven by low currents (-0.1 to -10 nA) of iontophoretically applied glutamate (10 mM in normal saline, pH 8).

Iontophoretically applied CCK-8 increased the firing rate of amygdaloid neurons tested. This uniform excitation is in marked contrast to hippocampal CA1 pyramidal neurons, which had only a 10-25% response rate and which frequently showed rapid desensitization to CCK-8. The enhanced firing rate induced by CCK-8 usually required the concurrent ejection of glutamate. For example, in the absence of ejected glutamate, CCK-8 at high ejection currents (to -40 nA) had no effect; but in the presence of glutamate which drove the otherwise silent neuron to fire, CCK-8 at low currents (-0.5 to -5.0 nA) markedly enhanced firing and could easily cause the cell to go into depolorization block. Furthermore, we have found that subthreshold currents of CCK-8 and glutamate (which by themselves had no effect on neuronal firing) significantly increased firing when both were applied together. In the nucleus centralis, the enhanced firing induced by CCK-8 could not be blocked by diazepam (1.2 mg/kg), or lorazepam (0.4 mg/kg), the putative CCK antagonist, proglumide (2.8 mg/kg), all given i.v. However, iontophoretically applied morphine sulfate markedly suppressed the enhancement of firing induced by CCK-8.

As CCK significantly enhanced the potency of concurrently administered glutamate, our results are most consistent with the role of CCK in the amygdala as a neuromodulator of the excitatory effect of glutamate.

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285.7 HUMAN PANCREATIC GROWTH HORMONE-RELEASING FACTOR (hpGRF), RAT GRF(1-29)$NH_2$, AND RAT GRF(3-40)OH ALTER THE ACTIVITY OF NEURONS IN RAT AMYGDALA. Michael J. Twery and Robert L. Moss. Dept. Physiology, Univ. Texas Hlth. Sci. Ctr., Dallas, TX 75235.

Extrahypothalamic regulation of growth hormone secretion is postulated to include inhibitory pathways which originate in the amygdala (1). Since nerve fibers containing GRF-like immunoreactivity have recently been reported to be in the amygdala, the present electrophysiological investigation was undertaken to study the effects of iontophoretically applied hpGRF, rat GRF(1-29)$NH_2$, and rat GRF(3-40)OH on the activity of individual amygdala neurons and to determine the local distribution of neurons which possess membrane sensitivity to these peptides. Experiments were performed on 15 male, Long-Evans rats (275-450g) anesthetized with urethane (1.3 mg/kg, IP). A multibarrelled glass microelectrode was used for extracellular recording of single units (center barrel, 4M NaCl, 4-12 MΩ) and iontophoretic application of chemicals.

Iontophoretic application of hpGRF altered the firing rate of 16 out of 24 amygdala neurons tested. Inhibition was the predominant effect (15 of 16). HpGRF depressed the firing rate in 5 of 5 central, 1 of 1 cortical, 2 of 3 medial, and 5 of 7 basolateral amygdala neurons, but only 2 of 8 lateral amygdala neurons. One neuron in the medial amygdala was excited by hpGRF. The firing rate of the 8 remaining neurons was not affected by the iontophoretic application of hpGRF. Further testing of 11 amygdala neurons determined that the majority (7 of 11) responded to rat GRF(1-29)$NH_2$ in a manner which resembled their response to hpGRF. Four neurons were inhibited and 3 neurons unaffected by application of either peptide. Some neurons responded only to hpGRF (N=3) or rat GRF(1-29)$NH_2$ (N=1). In 10 amygdala neurons the effect of hpGRF on neuronal firing rate was compared to that of rat GRF(3-40)OH. Iontophoretic application of rat GRF(3-40)OH decreased the firing rate of 5 neurons whose activity had been inhibited by hpGRF and produced no effect on the firing rate of 2 neurons which were also not affected by hpGRF. Three neurons exhibited membrane sensitivity to either hpGRF (N=2) or rat GRF(3-40)OH (N=1) but not both peptides. These results confirm earlier reports from this laboratory that a subpopulation of neurons in the rat CNS possess membrane sensitivity to hpGRF. Importantly, the present findings indicate a possible role for GRF in regulating amygdala efferents which have an influence on the hypothalamic control of GH secretion. We thank Drs. W. Vale and J. Rivier (Salk Institute) for peptides used in this study. This work was supported by NIH grant NS10434 to R.L.M. M.J.T. is an NIH postdoctoral trainee (HD07062).

1. Martin, J.B. (1976). The Brain Regulation of Growth Hormone Secretion. In: Frontiers in Neuroendocrinology, Vol. 4, L. Martini and W. Ganong (eds.), Raven Press, pp. 149-152.

285.8 ESTROGEN PRIMING EFFECTS LUTEINIZING HORMONE-RELEASING HORMONE (LHRH) SENSITIVITY BUT NOT β-ENDORPHIN (β-END) SENSITIVITY OF NEURONS IN THE MIDBRAIN CENTRAL GRAY (MCG). Mya C. Schiess*, Carol A. Dudley, and Robert L. Moss (SPON: W. Weinberg). Dept. Physiol., UT Health Sci. Ctr., Dallas, TX 75235.

The MCG is believed to modulate the lordosis response in the female rat by acting as an intermediate relay station between the descending input from the medial preoptic and ventromedial nucleus of the hypothalamus and its own output to the medullary reticular core. The present investigation determined the effect of exogenously administered estrogen (EB) and EB plus progesterone (P) on the membrane sensitivity of MCG neurons to LHRH and β-END. Experiments were completed on 26 female ovariectomized Sprague-Dawley rats (275-325 g) anesthetized with urethane (1.3 mg/kg, IP). All animals received 1 µg EB/week to maintain receptor integrity. Animals receiving this treatment only were classified as non-primed (n=6). EB-primed animals received 10 µg EB 48 hrs prior to recording (n=11) and EB-P animals received 10 µg EB at 0 hrs and 2.5 mg P at 48 hrs (n=9). Seven-barreled glass microelectrodes were used for single unit extracellular recording (center barrel, 4M NaCl, 6-10 MΩ) and iontophoresis of LHRH (1 mM, pH 7) and β-END (1 mM, pH 7.1). Cell localization was determined by ejecting pontamine sky blue and histologically reconstructing the electrode tracks.

Iontophoresis of LHRH increased the firing rate in 31 cells and decreased the firing rate in 33 cells from a total of 94 cells tested, while iontophoretic application of β-END excited only 22 and inhibited 40 of the same 94 cells. Application of LHRH and β-END elicited no change in the firing rate of 29 and 26 of the 94 cells respectively. In the non-primed rat, the majority of cells were inhibited by LHRH (14 of 23), while a smaller number were excited (6) or showed no response (7). In contrast, in animals primed with EB, the predominant effect of LHRH was excitation (17 of 34); 7 cells were inhibited and 10 showed no change. In EB-P animals, LHRH produced a response profile similar to that obtained in non-primed animals in that more cells were inhibited (12 of 33) than excited (8 of 33) by the decapeptide. The percent of cells responding to β-END with excitation, inhibition, or no response was not altered by hormonal priming paradigms. Additionally, a discrete area of the MCG at the level of the superior colliculi in a dorsal medial region consistently showed the greatest number of cells with membrane sensitivity to LHRH and β-END.

These results suggest that EB priming can alter the membrane sensitivity to LHRH of neurons in the MCG, and that these effects appear to be negated by the addition of P. Importantly, these findings suggest a possible MCG neurohumoral mechanism for modulating the lordosis reflex, whereby estrogen increases an LHRH induced excitation at the MCG which in turn may contribute to the facilitation of the lordosis response. Supported by NIH HD00615 and NS10434.

285.9 PRESENCE AND FUNCTIONAL SIGNIFICANCE OF CALCITONIN GENE RELATED PEPTIDE (CGRP) IN THE CEREBRAL CIRCULATION. T.A. Kingman*, L. Edvinsson*, R. Ekman*, R. Uddman* and J. McCulloch. Wellcome Surgical Institute, University of Glasgow, United Kingdom, and the University of Lund, Sweden.

The 37 amino acid peptide, calcitonin gene related peptide (CGRP) has recently been identified, in neuronal tissue, as an alternative expression product of the calcitonin gene. We have established the presence and origin of CGRP-immunoreactivity in nerve fibres around cerebral vessels and have characterised the vasomotor actions of the peptide upon cerebral vessels in vitro and in situ.

Dense plexuses of CGRP-immunoreactive nerve fibes invest the major cerebral arteries and the pial arterioles of the cortical surface. These nerve fibres are present in the adventitia and at the adventitial-medial border in the blood vessels. The CGRP-like immunoreactive material in feline cerebral blood vessels appears to be extremely similar, or identical to rat CGRP on HPLC. There are numerous CGRP-immunoreactive perikarya in the feline trigeminal ganglia. Unilateral surgical division of the trigeminal nerve under anaesthesia 14 days prior to sacrifice resulted in the loss of CGRP-like immunoreactivity from ipsilateral cerebral vessels. In the trigeminal ganglia and cerebrovascular nerve fibres, CGRP-like and substance P-like immunoreactivity are co-localised.

Rat CGRP is a potent dilator of cat middle cerebral arteries in vitro (max. relaxation: 10.5 $\pm$ 1.5 mN; $EC_{50}$ 9.6 $\pm$ 1.3 nM) and of the cortical arterioles of anaesthetised cats in situ (max. increase in calibre: 38 $\pm$ 5% at perivascular microapplication of CGRP $10^{-7}$M). The arteriolar dilatations produced by high concentrations of CGRP were markedly persistent, with arterioles remaining dilated for 5-10 minutes. Both in vitro and in situ, CGRP is the most potent cerebrovascular dilator neuropeptide yet tested.

The physiological significance of cerebrovascular trigeminal CGRP system was investigated after chronic (14 day) surgical lesions of the trigeminal nerve. The cortical arteriolar responses to subarachnoid microinjections of acidic (pH 6.8) and basic CSF (pH 7.6) were examined in anaesthetised cats with immunocytochemically verified lesions of the trigeminal nerve, and contrasted with their effects in sham-operated animals. The magnitude of the vasodilatory and vasoconstrictor responses to these agents was unaffected by trigeminal lesions. However, the duration of the vasoconstriction produced by basic CSF, but not the vasodilatation to acidic CSF, was markedly prolonged by trigeminal lesions (from 0.8 $\pm$ 0.1 to 2.2 $\pm$ 0.3 min, $p < 0.01$). The cerebrovascular trigeminal system, in which CGRP is the most potent vasoactive constituent, appears to be a neurogenic mechanism which provides protection to the brain against excessive vasoconstriction.

285.10 EFFECTS OF DAILY TRH ADMINISTRATION IN MURINE MOTOR NEURON DISEASE (Wobbler Mice) W. Kozachuk*, H. Mitsumoto, V. Salanga*, A. Boggs*, (Spon: R.J. Lederman)Department of Neurology, Cleveland Clinic, Cleveland, OH 44106 and J. Wilber, Endocrinology, L.S.U., New Orleans, LA

Although the neurobiological function of TRH found in axon terminals apposing spinal cord anterior horn cells has yet to be elucidated, the clinical use of TRH for the treatment of human motor neuron disease has been advocated (Engel et al, 1983). We tested the effects of TRH on neuromuscular functions in the wobbler mouse, the best natural disease model for the investigation of human motor neuron disease. Wobbler mice develop their disease at the age of 3-4 weeks with rapidly progressive paralysis, atrophy and contractures in the forelimb muscles. At the time of clinical detection, intraperitoneal injection of 50 mg/kg of TRH (the dosage producing compulsive sustained scratch behavior, jumping and jitteriness lasting 10-15 minutes after the injection) was given daily (6 days a week) for 9 weeks in 5 wobbler mice and 5 healthy littermates. Another group (5 pairs) received saline-placebo injections identical to the TRH group. Weekly assessments were performed by 2 examiners (VDS and HM) without knowledge of drug identification, including body weight, time running $2\frac{1}{2}$ feet (best of 3 trials), front paw grasping power (best of 3 trials by means of gram dynamometer), and semiquantitative grading for gait features, eye hygiene, grooming, and the degree of forepaw contractures. At the end of the treatment the brain and spinal cord were sampled for TRH and cyclo(His-Pro) determinations. Control animals with TRH did not develop deleterious effects on weight increase, or other neuromuscular functions. In wobbler mice all neuromuscular function steadily declined regardless of the type of treatment. Although TRH-treated wobbler mice appeared slightly better in running time and grasp power there was no statistical significance between the two groups. Semiquantitative tests also showed no differences. TRH levels in the spinal cord were found to be significantly increased ($p<0.01$) in placebo-treated wobbler mice (252.9 $\pm$ 23.9 pg/mg protein) as compared to controls (102.1 $\pm$ 17.7 pg/mg protein). However, there were no significant differences in TRH and cyclo(His-Pro) levels in TRH-treated animals. The lack of beneficial effects with TRH in wobbler mice may be due to the increased TRH levels in the wobbler spinal cord; hence further administration of TRH has no additional effect. The negative results from the TRH trials in murine motor neuron disease supports the negative results found in our TRH trials with patients with amyotrophic lateral sclerosis (Mitsumoto et al, 1984 and 1985). (The study was partly supported by the A.L.S. Society of America)

285.11 FAILURE OF SUBSTANCE P ANALOGUE, SPANTIDE, TO ANTAGONIZE GASTRIC EFFECTS OF BOMBESIN. Y. Tache, M. Gunion, D. Hamel*, T. Pappas* and H. Debas*. Center for Ulcer Research and Education, VA Wadsworth Med. Ctr., Dept. of Medicine, UCLA, Los Angeles, 90024.

Spantide [D-Arg1,D-Trp7,9,Leu11] substance P has been reported to be a specific and potent antagonist of the binding of bombesin peptides and substance P to exocrine pancreas and brain receptors (Yachnis et al., Life Sci. 35:1963-1969, 1984). The ability of spantide to antagonize bombesin induced changes in gastric secretion was investigated in rats and dogs. Rats fasted for 24 h were injected under ether anesthesia either intracisternally (ic) in 10 ul volume with spantide or saline and 15 min later with bombesin or intracerebroventricullarly (ivt) in 5 ul volume with bombesin alone or combined with spantide, then the pylorus was ligated. Rats regained consciousness within 5-10 min after the injection. The animals were decapitated 2 h after pylorus ligation and their gastric secretion collected. Bombesin given ic (6.2 pmol) or ivt (62 pmol) inhibited gastric secretion. Spantide (2.0 nmol ic, or 6.6 nmol ivt) did not modify bombesin action. The ic route of administration of spantide was associated with 100% mortality within 1-10 min at peptide dose of 3.3 nmol and 58% at 2.0 nmol dose. Injection of spantide icv (6.6 nmol) did not cause mortality but elicited severe barrel rolling in all rats. In conscious mongrel dogs with gastric fistula, intravenous infusion of bombesin (6.2 pmol/kg.h) stimulated gastric acid and gastrin secretions. Spantide infused intravenously (10 nmol/kg.h) starting 30 min prior to bobmesin and throughout the experiment did not alter either gastrin or gastric acid response to bombesin. No behavioral changes were observed. In summary 1) in rats, nmol doses of spantide injected into the cisterna magna are lethal; given ivt, the peptide elicited severe barrel rolling 2) spantide did not antagonize the central or peripheral actions of bombesin on gastric secretion. These data indicate that the use of spantide as bombesin antagonist in vivo is limited.
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285.12 CHOLECYSTOKININ OCTAPEPTIDE (CCK-8) IS RELEASED FROM THE HYPOTHALAMUS IN RESPONSE TO AN INTRAGASTRIC MEAL IN ANESTHETIZED CATS. R.R. Schick*, T.L. Yaksh*, V.L.W. Go*. (SPON: J.D. Grabow) Gastroenterology Unit and Neurosurgical Research Unit, Mayo Foundation, Rochester, MN 55905.

CCK-8 suppresses feeding in several species after either peripheral or central administration and has therefore been considered as a putative satiety factor. The presence of CCK-8 in the hypothalamus, a brain area involved in feeding regulation, suggests a hypothalamic site of action.

Aim: The present study was designed to investigate the physiological significance of these findings by examining if hypothalamic extracellular levels of CCK-8 are influenced by a test meal.

Materials and Methods: In 5 halothane anesthetized cats (2.5-4 kg) push-pull perfusions of the lateral hypothalamus were carried out stereotaxically (AP 12.5/LAT 3.0/HV -1.0) as described previously (J Appl Physiol 37:428, 1974). Perfusion samples were collected in 30-min intervals. Peripheral venous blood samples were frequently drawn for gastrin measurements. After a 90-min baseline perfusion, a test meal consisting of 4 g carbohydrates and 4 g amino acids dissolved in 30 ml water was administered intragastrically. 2 hrs postprandially a 30-min perfusion with 40 mM KCl was performed additionally. In 3 cats intravenous (i.v.) infusions of synthetic CCK-8 at increasing rates of .05, .2, 2 and 20 µg/kg · h, each lasting 30 min, were carried out to assess the existence of a blood-brain barrier. Plasma gastrin and perfusate levels of CCK-like immunoreactivity (CCK-LI) were determined by radioimmunoassay. Identification of the molecular form of hypothalamic CCK-LI was performed with reverse phase high performance liquid chromatography (HPLC).

Results: Baseline CCK-LI in the hypothalamic push-pull perfusate was below assay sensitivity (< 7 pg/30 min). After the test meal, however, CCK-LI rose to 27$\pm$3 pg/30 min and 24$\pm$4 pg/30 min, respectively in the first two postprandial perfusion samples thereafter falling below assay sensitivity again. This rise in perfusate CCK-LI was accompanied by an increase of peripheral venous gastrin levels from a mean baseline of 233$\pm$24 pg/ml to a maximum of 320$\pm$43 pg/ml after 10 min. During potassium perfusion, perfusate CCK-LI rose again up to 27$\pm$11 pg/30 min. HPLC characterization demonstrated that the majority of CCK-LI coeluted with the synthetic CCK-8 standard. During i.v. infusion of synthetic CCK-8 peripheral venous plasma levels of CCK-LI were increasing from 94$\pm$37 pg/ml by 2 log units, but hypothalamic perfusate levels of CCK-LI did not change.

Conclusion: These data show that extracellular levels of CCK-8 are increasing in the lateral hypothalamus after administration of a meal and support the hypothesis that CCK-8 may play a physiological role in the central regulation of feeding and satiety.
Supported by NIH grant AM34988, and DFG, Germany (RRS).

285.13 CHARACTERIZATION OF THE NEURALLY-INDUCED INCREASE IN VASCULAR PERMEABILITY IN RAT TRACHEA. J.J. Brokaw and D.M. McDonald. Cardiovascular Research Institute and Department of Anatomy, Univ. of California School of Medicine, San Francisco, CA 94143

Electrical stimulation of the cervical vagus nerve is known to increase the permeability of blood vessels in the rat trachea. Current evidence is consistent with the hypothesis that this response is mediated by substance P released from unmyelinated vagal afferents in the airway mucosa. The aim of our study was to characterize the response by determining the rate of onset and duration of the change in vascular permeability and to assess the development of tachyphlaxis to subsequent stimuli.

In anesthetized rats treated with atropine (1 mg/kg, i.v.), the right vagus nerve was cut rostral to the superior laryngeal branch and then stimulated electrically coincident with the injection of Evans blue dye (30 mg/kg, i.v.). Five min later, the rats were perfused with 1% paraformaldehyde in citrate buffer (pH 3.5) and the tracheas removed. Tracheal dye-content was measured by spectrophotometry. The amount of dye extravasation was found to vary with the duration, frequency, and voltage of the stimulus. The conditions producing the maximal effect were found to consist of a 15 sec stimulus of 5 V at 20 Hz; these conditions were used in our other experiments.

To explore the kinetics of the response, intervals of time between the electrical stimulus, the dye injection, and the fixative perfusion were systematically varied in different groups of rats over the range of 0 - 10 min. The results indicate that the dye content of the trachea was half-maximal at 1 min and maximal by 5 min. The increase in vascular permeability had a half-life of 2 min. The time-course of tachyphylaxis was investigated by varying the interval between two stimuli 2 min to 4 hr apart. The results showed that within 10 min of the first stimulus, there was a diminished response to the second stimulus.The degree of tachyphylaxis was less after an initial stimulus of 15 sec duration than after one of 5 min. The subnormal response to the second stimulus persisted for 2 - 4 hr after the first stimulus.

In summary, the neurally-induced increase in vascular permeability in the rat trachea depends upon the duration, frequency, and amplitude of the stimulus. It has a rapid onset and a short duration, and is subject to a rapidly developing tachyphylaxis that is long lasting. This project was supported by NIH training grant HL-07185 and program project grant HL-24136.

## CHEMICAL SENSORY SYSTEMS I

286.1 ADEQUATE STIMULI FOR HYDROXY-PROLINE RECEPTORS IN THE OLFACTORY ORGAN OF THE AMERICAN LOBSTER. B.R. Johnson, C.L. Merrill* and J. Atema. Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543.

The lateral antennule from the American lobster, Homarus americanus, functions as an olfactory organ; it is necessary for efficient orientation to a food odor source (Devine, D.V. and Atema, J., Biol. Bull., 163:144, 1982). A majority of the chemoreceptors surveyed from the lateral antennule of H. americanus are narrowly tuned to trans-4-hydroxy-L-proline (OH-Pro) (Johnson, B.R. and Atema, J., Neurosci. Letts. 41:145, 1983). This suggests that OH-Pro may act as a chemical feeding signal. However, OH-Pro is usually bound in connective tissue proteins of lobster prey and thus may be normally unavailable to stimulate chemoreceptors as a free amino acid. Here we examined the tuning of OH-Pro cells from lateral antennules with a variety of compounds to determine the adequate stimuli for this receptor population. We chose compounds suspected of possible social functions or associated with breakdown of connective tissue.

Single OH-Pro sensitive cells were identified through extracellular recordings from small bundles of the antennular nerve in ablated lateral antennules. Cells sensitive to $10^{-5}$M OH-Pro were then tested for their sensitivity to proline, kainate, octopamine, serotonin, hydroxy-lysine, cis-4-hydroxy-D-proline, and cis-4-hydroxy-L-proline, all at $10^{-5}$M; alpha and beta ecdysone at $2x10^{-6}$M and gelatin in a fully saturated and one-tenth diluted solution. Gelatin is denatured connective tissue which contains 14% bound OH-Pro. Most of the test compounds elicited little or no response. Cis-4-hydroxy-D-proline and cis-4- hydroxy-L-proline were not stimulatory, demonstrating the stereo-specificity of these chemoreceptor responses. Serotonin and particularly gelatin caused strong responses in some of the cells tested.

The responses to OH-Pro, gelatin and serotonin suggest that these or similar compounds may play a role in feeding and/or social behaviors of the lobster. The responses to gelatin may indicate that these receptors are tuned to collagen and its breakdown products. Studies are in progress to further characterize the OH-Pro receptor response to serotonin, collagen and collagen derivatives.

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286.2 AMILORIDE BLOCKS THE BEHAVIORAL FEEDING RESPONSE BUT NOT THE NERVE RESPONSE TO FEEDING STIMULI IN THE LEECH. E. J. Elliott. Dept. of Zoology, Univ. of Maryland, College Park, MD 20742.

Studies of chemoreception in the leech Hirudo medicinalis have shown that Hirudo can detect and will ingest artificial mixtures of chemicals found in blood. In particular, a mixture containing only NaCl (150 mM) and arginine (0.1 to 1 mM), but not NaCl alone or arginine alone, is recognized and ingested as readily as blood. This mixture also elicits a robust response in the cephalic nerves innervating the dorsal lip sensilla, chemosensory structures located near the mouth. The diuretic drug amiloride, which at micromolar concentrations inhibits passive $Na^+$ transport and at higher concentrations blocks $Na^+$-$H^+$ exchange in transport epithelial cells, has been shown in a variety of species also to inhibit chemosensory or taste responses to NaCl, by behavioral and/or electrophysiological criteria. Bathing a leech in amiloride reversibly blocked the feeding response (i.e., attraction, probing, attachment and ingestion) to the mixture of NaCl + arg, as well as to blood. $10^{-4}$M amiloride inhibited the response of 50% of the leeches tested, and $10^{-3}$M inhibited feeding in all leeches tested. These results suggest that, as proposed for other systems, amiloride blocks the chemosensory detection of NaCl by the leech. However, in an isolated lip preparation in which the outside surface of the lip was bathed in amiloride, the response recorded extracellularly from the cephalic nerves during stimulation of the lip sensilla with NaCl + arg remained unchanged. Possible explanations for the apparent discrepancy in these results are being explored. The nerve response to NaCl + arg is more complex than simply the sum of the response to NaCl and the response to arginine. Moreover, the behavioral inhibition by amiloride may be a non-specific effect unrelated to a block of $Na^+$ transport or NaCl sensing. Supported by NIH grant NS 20432.

286.3 ELECTRICAL PROPERTIES OF OLFACTORY CELLS ISOLATED FROM THE EPITHELIUM OF THE TIGER SALAMANDER. Stuart Firestein* and Frank Werblin* (SPON: P.A. Anderson). Graduate Group in Neurobiology, University of California, Berkeley, CA 94720.

Enzymatically dissociated olfactory epithelial cells were studied using the 'gigaseal' whole cell patch clamp technique. Cells identified morphologically as neurons had a resting potential of -45 mV and an input resistance between 2.5 and 5 gigohms. This value is more than an order of magnitude greater than that reported previously using intracellular microelectrodes.

Two unusual transient inward currents could be identified. The larger current, up to 1.0 nA in magnitude, was TTX insensitive but blocked by the addition of $Co^{2+}$ and is presumed to be carried by $Ca^{2+}$ ions. This current activates over a narrow potential range between -40 and -30 mV and inactivates within 20 msec. A second transient inward current was blocked by TTX; it is less than 0.5 nA and shares a similar time course and activation range with the transient $Ca^{2+}$ current. There was no sustained inward current.

Two outward currents, both carried by $K^+$, could be distinguished kinetically and pharmacologically. One was an inactivating current which was $Ca^{2+}$ dependent and closely followed the transient $Ca^{2+}$ current; the other was sustained and voltage dependent. Both currents could be blocked by replacing KCl with CsCl in the patch electrode.

The unusually high input resistance measured in these cells would allow even small transduction currents to polarize the membrane to within the activation range of the gated currents. Thus, these gated currents could be actively involved in forming the cell's response waveform.

286.4 A CELL FREE ASSAY FOR OLFACTORY REACTIVITY REVEALS PROPERTIES OF ODORANT RECEPTOR MOLECULES. Zehava Chen*, Michael Greenberg*, Umberto Pace* and Doron Lancet (Spon: J.M.Gershoni) Dept. of Membrane Research, The Weizmann Institute of Science,Rehovot,Israel

We have recently reported that isolated olfactory cilia exhibit odorant-stimulated, GTP-dependent cyclic AMP synthesis (Pace,U., Hanski,E., Salomon,Y. and Lancet D. Nature, in press; Lancet et al, this volume). This strongly suggests that olfaction is mediated by receptor-coupled cyclic nucleotide processing enzyme and a GTP-binding protein (G-protein), similar to visual, hormone and neurotransmitter reception. We utilize the measurement of cyclic AMP production as a novel cell-free assay for olfactory reactivity, useful in studying and possibly identifying olfactory receptor proteins. We find that both in-vivo electroolfactogram responses and the cell-free elevation of cyclic AMP synthesis induced by various odorants are specifically diminished in the presence of the lectin wheat germ agglutinin (WGA), or by the sulfhydryl-blocking reagent N-ethyl maleimide (NEM). Such correspondence between in-vivo and in-vitro results supports the notion that ciliary adenylate cyclase activity is related to physiological olfactory responses. In parallel, these findings provide new information on the putative olfactory receptor molecules. The only frog ciliary protein that reacts with WGA is gp95, a major glycoprotein that fulfills several criteria for being a membrane receptor molecule (Chen et al, PNAS 81, 1859 (1984)). The polypeptide gp95 is glycosylated, spans the membrane, is enriched in the sensory organelles and its abundance and molecular size correspond to those of ciliary freeze fracture intramembranous particles, as expected for olfactory receptor proteins. The WGA inhibition constitutes an additional important indication for the possible functional role of gp95. The effect of NEM on cell-free odorant-induced cyclic AMP synthesis is found to be mainly due to direct inhibition of adenylate cyclase: at an NEM concentration where 95% of the cyclic AMP production is lost, the relative stimulation by odorants remains virtually unchanged. Thus, it appears most plausible that NEM affects functionally important fulfhydryls in the transductory enzyme rather than in the receptor molecule itself. In view of results obtained in other systems, such a model would still be consistent with odorant protection against NEM inhibition - via an allosteric mechanism. Currently we are pursuing reconstitution experiments aimed at transferring odorant receptors from ciliary membranes to other membranes, where adenylate cyclase is odorant-insensitive. Such studies will provide a direct means for identification and eventual isolation of olfactory receptor proteins.

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286.5 SIMPLE PATTERN RECOGNITION MODELS OF OLFACTORY DISCRIMINATION. T. J. Sejnowski, P. K. Kienker* & G. M. Shepherd. Biophysics Dept., Johns Hopkins Univ., Baltimore, MD 21218 & Sect. of Neuroanatomy, Yale Univ. Sch. of Med., New Haven, CT 06510

In a previous $^{14}$C-2-deoxyglucose study of the rat olfactory bulb several different odors were presented over a range of concentrations and the patterns of labeling observed in the glomerular sheet where the receptor axons terminate (Stewart et al., J. Comp. Neur. 185:715, 1979). The patterns, though different, showed significant overlap for all three odors tested as well as the pure air control. We have generated several network models to see whether odors could be discriminated on the basis of these two-dimensional patterns of activity within the glomerular sheet of the bulb.

The activity pattern for each of the four odor conditions was approximated by contour plots on an 8x8 grid. The highest values represented the most sensitive regions for that odor. An olfactory "unit" was assigned to each of the 64 grid regions and for a particular "sniff" the input pattern was generated by taking each unit to be active or not with a probability proportional to its sensitivity and to the log concentration.

In the first network four additional units were chosen to represent the four odors each of which had a connection to all 64 olfactory units. Each unit summed the active units in a given sniff weighted by the synaptic strength, and if the total was above threshold the unit was considered active. Following each sniff the weights were changed according to the perceptron learning rule. After 2,000 sniffs the weights converged to a stable pattern. The overall performance of the network was 90% correct discrimination at high concentrations with a gradual falloff to chance level at low concentrations.

In a second model a layer of 12 additional units was interposed between the 64 olfactory units and the 4 odor units. The same training procedure was followed except that the Boltzmann learning rule was applied to all the weights between the layers of units (Ackley et al., Cog. Sci. 9:147, 1985). The weights in this network converged after 10,000 sniffs to a pattern in which all of the 12 "feature" units had excitatory and inhibitory connection to more than one odor unit. The performance was 97% at high concentrations and about 25% better than the previous model at low concentrations; the performance was also much less affected when random changes were made to the weights.

These simulations provide examples of networks that can discriminate between different odors using only the information in the spatial pattern of activity at the level of sensory input to the olfactory bulb despite the large overlaps between the patterns of different odors. Some odor discriminations could be acquired in this manner even though the initial connectivity may be innate.

286.6 THE PRIMARY OLFACTORY PROJECTION HAS TWO CHEMICALLY DISTINCT ZONES. J.E. Schwob and D.I. Gottlieb. Dept. Anatomy & Neurobiology, Washington University School of Medicine, St.Louis, MO 63110.

The receptor neurons of the olfactory epithelium are an anatomically uniform population which are differentially excited by odorants. We have discovered an unexpected chemical heterogeneity in this population. A newly discovered monoclonal antibody, designated RB-8, stains about 2/3's of the olfactory epithelial sheet and its projection onto the olfactory bulb. The remaining 1/3 is either unstained or stained at a much lower level.

RB-8 is an IgG derived from a fusion of SP-2/0 myeloma cells with splenocytes from a mouse immunized 4X with triturated homogenates of neonatal rat posterior piriform cortex. The RB-8 producing hybridoma was cloned twice and found to be a stable producer of antibody over a period of several months of continuous growth. Binding of unlabelled primary antibody to 8-12 µm thick cryostat sections of the rat primary olfactory projection was visualized with the Vectastain ABC reagents (Vector Laboratories).

In sections of decalcified muzzle from adult rats, RB-8 stains the receptor neurons of the ventral and lateral parts of the olfactory epithelium and densely labels fascicles of the olfactory nerve projecting from this RB-8 positive area. RB-8 labelling of the epithelium of the dorsal recess and medial tips of the dorsal turbinates is not detectable, and the fascicles from this RB-8 negative area are only very weakly stained. These RB-8 negative areas form a contiguous zone on flattened maps of the epithelial sheet. RB-8 staining of the glomeruli in the ventrolateral part of the bulb is correspondingly heavy, while that in the dorsomedial glomeruli is undetectable or weak. In RB-8 labelled glomeruli, the staining is precisely co-extensive with anti-olfactory marker protein staining. In addition, knife cut lesions of the olfactory nerves totally eliminate the RB-8 staining in the glomeruli where the destruction of the olfactory terminals is complete. We conclude that in the olfactory nerve and the glomeruli, RB-8 is staining the olfactory axons and their terminals. Survey of the CNS and peripheral tissues demonstrates that staining with RB-8 is nervous system specific; however, not all components of the CNS and PNS are stained.

In conclusion, these results demonstrate that the primary olfactory projection can be visualized as a sheet which is divided into two portions; one of these is stained with RB-8 and the other is not. These two portions may have distinguishable roles in early development and/or olfactory sensation. A similar result was recently reported for the rabbit (Fujita et al., Brain Res. 326:192-196, 1985).

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286.7 MAPPING RECEPTOR POPULATIONS THAT PROJECT TO GLOMERULI IN THE MAIN OLFACTORY BULB. P. E. Pedersen, P. J. Jastreboff*, W. B. Stewart and G. M. Shepherd. Sec. of Neuroanatomy, Anatomy, and Neurosurgery, Yale Univ. Sch. of Med., New Haven, CT 06510.

Spatial patterns of activity in the glomeruli of the olfactory bulb in response to odor stimuli have suggested the hypothesis that there is a spatial organization of olfactory receptor neurons projecting to those glomeruli (Stewart et al., 1979). We have introduced the horseradish peroxidase (HRP) tracing technique to identify populations of olfactory receptor cells that project to specific groups of glomeruli (Jastreboff et al., 1984). The aim of the present study was to develop a method for mapping these HRP-labeled populations of receptor neurons within the nasal cavity of young rats.

Ionophoretic injections of wheat germ agglutinin HRP were made via stereotaxically placed glass micropipettes into the modified glomerular region of the MOB. Pups (N=21) were treated according to procedures reported previously (Jastreboff et al., 1984). Camera lucida drawings were made of the entire olfactory bulb and the nasal cavity of six experimental animals. In order to establish the relationship between the bulb and its receptor sheet, maps of the entire olfactory epithelium were constructed in 2 dimensions; within these maps, the distribution of labeled neurons was plotted, and compared with maps of the location of HRP injected into the olfactory bulbs.

HRP label was confined to a clearly identifiable localized region in the modified glomerular region of the MOB. Label included 2-9% of the glomerular sheet of the MOB, including the modified glomerular complex. HRP labeled receptor neurons within the olfactory epithelium ipsilateral to the injected side occurred within topographically defined regions. Anteriorly, labeled olfactory neurons were confined to a narrow strip in the dorsal recess, and, more posteriorly, this strip widened medially along the septal wall and laterally onto a limited area on the nasal turbinates. In very posterior regions most of the epithelium was occupied with labeled cells. The labeled olfactory neurons were confined to 9-32% of the total epithelial area; in addition, they comprised only a portion of the receptor population within a region. This suggests that the unlabeled neurons project to other glomerular regions of the MOB. The functional implications of these findings will be discussed as well as analysis of HRP label at the cellular level.

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286.8 THE TOPOGRAPHY OF OLFACTORY EPITHELIUM TO OLFACTORY BULB PROJECTIONS IN THE RAT. William B. Stewart, Patricia E. Pedersen, Charles A. Greer and Gordon M. Shepherd. Sections of Anatomy, Neurosurgery, Neuroanatomy, Yale University School of Medicine, New Haven, CT. 06510.

We have shown previously, using the 2-deoxyglucose method, that specific regions of the olfactory bulb were metabolically active during exposure to odors. Furthermore, different regions were active with different odors. We have also shown, using the horseradish peroxidase (HRP) method, that one of the metabolically active regions in the posterior medial olfactory bulb receives projections from a defined region of the olfactory epithelium. The present experiments were designed to test the hypothesis that another metabolically active region in the lateral anterior olfactory bulb receives its input from a different region of the olfactory epithelium. Therefore, rats had HRP placed in restricted regions of the olfactory bulb by iontophoresis or by direct placement of HRP crystals. The distribution of HRP in the decalcified nose was examined following sacrifice and processing. Placement of HRP in the medial posterior bulb produced labelled receptor cells along the septal wall, posterior dorsal recess and medial turbinals. By contrast, placement of HRP in the anterior lateral bulb produced labelled receptors along the dorsal and lateral turbinals and the anterior dorsal recess, but not the septal wall. These results show that these two regions of the bulb receive input from distinct but overlapping populations of receptor neurons.

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286.9 HISTOLOGICAL LOCALIZATION AND IMPROVED RESOLUTION OF 2-DEOXYGLUCOSE UPTAKE IN THE OLFACTORY BULB USING COMPUTERIZED IMAGE PROCESSING. J.S. Kauer. Depts. of Neurosurgery, Anatomy and Cell Biology, Tufts-N.E.M.C., Boston MA 02111

The 2-deoxyglucose (2DG) method has been widely used to map regional brain glucose metabolism under a variety of experimental conditions. In its usual format the method provides a view of glucose uptake related to neuronal activity throughout the brain with a practical resolution, dependent on a number of factors, of 50-100 μm. Autoradiographs are usually generated on X-ray film which is separated from the tissue sections at the time of film development. In order to analyze which anatomically defined brain structures show changes in glucose uptake, the densities on the autoradiographs must be compared to the original tissue sections. Several systems have been developed for this prupose (Gallistel et al., Neurosci. Biobehav. Rev. 6:409, 1982; Kauer et al., J. Neurosci. Meth. 11:143, 1984). Here we present a computerized image processing method for superimposing the image of the stained histological section on its companion autoradiograph allowing exact correlation of architectonic structure and glucose uptake densities. The method consists of storing a digitized 512 x 512 x 8 bit image of an autoradiograph, giving a possible range of 255 gray levels for each pixel in the computer memory and aligning with it, on the TV monitor, its histological counter-part. The image of the autoradiograph is then retained in memory in registry with the histology, but blanked from view, leaving only the histological image visible. Single density values on the hidden autoradiograph may then then be assigned a color which is superimposed onto the histological image. By looking at the sites of color-enhanced single gray levels, minute differences in glucose uptake may be observed that are directly related to the cellular architecture, thus providing higher practical resolution than other image processing methodologies.

Previous experiments in which glucose metabolism was examined in the olfactory bulb (Sharp et al., Br. Res. 107:663, 1975; Stewart et al., J. Comp. Neurol. 185:715, 1978) have shown increased 2DG uptake in glomerular layer foci in response to odor. Using the method described here, we have often found that highest 2DG uptake appears in the periglomerular regions coextensive with slightly lower uptake within the glomeruli, which together make up the dense foci seen in previous studies. These observations suggest that 2DG uptake is manifest in periglomerular cell bodies which are more active during odor stimulation than intraglomerular structures. Localized uptake has also been seen related to activity in mitral, tufted and granule cells as well as various regions of neuropil. Supported by NINCDS NS-20003.

286.10 TOPOGRAPHICAL PROJECTIONS OF SEPTAL ORGAN RECEPTOR NEURONS TO THE MAIN OLFACTORY BULB IN RATS. T.E. Benson, P.E. Pedersen, P.J. Jastreboff* and G.M. Shepherd. Section of Neuroanatomy, Yale University School of Medicine, New Haven, CT 06510-8001.

The septal organ of Masera (SO) is an isolated patch of olfactory epithelium. Its relatively simple topology makes it suited to a study of the topography of olfactory receptor neuron projections into the olfactory bulb. As a component of our ongoing studies on receptor-bulb relations we have mapped the distribution of labeled receptor neurons in the SO following punctate injections of horseradish peroxidase (HRP) into the olfactory bulb. We have thus tested the hypotheses that SO receptor neurons project to the ipsilateral main olfactory bulb and that this projection is topographically organized.

Wheat germ agglutinin HRP was ionophoretically delivered into the dorso-caudo-medial olfactory bulb of 10 young rats and tissues processed as previously described (Jastreboff et al, PNAS 81: 5250, 1984). Injection sites comprised 2-8% of main olfactory bulb glomeruli. Camera lucida drawings were made of SO and nearby landmarks at 25x. Sagittal maps were generated for qualitative assessment of labeled receptor neuron distributions and quantitative analyses of distributions were performed.

In 5 animals SO receptor neurons ipsilateral to the injected olfactory bulb were retrogradely labeled. The labeled cells had a morphology similar to that of olfactory receptor neurons in the main olfactory epithelium labeled by retrograde transport of HRP from the olfactory bulb (Ibid). The epithelium of the SO was thinner than the main olfactory epithelium, especially in its dorsal part.

Correlation of bulbar injection sites with the distribution of labeled receptor neurons within the SO indicated a distinct topography in the projection of the SO into the olfactory bulb. Labeled receptor neurons were preferentially located in the caudal SO and were clustered about a plane dorsal to the plane bisecting the SO. Animals with injection sites confined to the accessory olfactory bulb had dense labeling of receptor neurons in the ipsilateral vomeronasal organ but no main olfactory epithelium or SO receptor neurons were labeled.

These results indicate that the projection of septal organ receptor neurons is ipsilateral and is most likely confined to the main olfactory bulb. They also indicate that the projection to the dorso-caudo-medial olfactory bulb does not arise randomly throughout the septal organ, rather there is clearly a degree of topographical organization in this projection. Because of the simple topology of the septal organ within the nasal cavity, the functional significance of this topographical organization invites further study.

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286.11 NADPH-DIAPHORASE AND NPY LABELING OF RAT OLFACTORY BULB SHORT AXON CELLS AND GLOMERULI. John W. Scott, John K. McDonald and Janice L. Pemberton*, Department of Anatomy, Emory University School of Medicine, Atlanta, Georgia 30322.

Short axon cells of the olfactory bulb are found in all of the cell layers of that structure but they exist in several classes with restricted distributions. At present no function has been clearly ascribed to these cells but they have been studied with morphological and immunohistochemical procedures. We have seen two populations of short axon cells that are intensely stained for the presence of NADPH-diaphorase and neuropeptide Y (NPY) in 80 micrometer serial sections of the rat olfactory bulb. The superficial short axon cell population corresponded to that observed with Golgi impregnation. Their somata lay near the border of the periglomerular region with the external plexiform layer. The dendrites were distributed in the periglomerular region and were never seen to enter the glomeruli. Multiply branching NPY immunoreactive axons from these cells could occasionally be traced across the upper portion of the external plexiform layer. A second population of short axon cells stained by both procedures was seen in the depths of the granule cell layer and the deep white matter surrounding the ventricular layer. These deep cells might correspond to either the Golgi or Blanes cells (Schneider and Macrides, Brain Res. Bull. 3:73-82, 1978) but in contrast to the Golgi observations or Blanes cells, no spines were apparent in our material. The deep short axon cells we observed are similar in appearance and distribution to those labeled by somatostatin immunohistochemistry (Davis et al., JCN 204:377-383, 1982). However, peptide or transmitter histochemistry of the superficial short axons cells has not been previously reported. Some olfactory glomeruli and the entering olfactory nerves were intensely stained with the NADPH-diaphorase procedure. We found that, while the intense NADPH-diaphorase staining of short axons cells and other neurons of the forebrain was abolished by periodate in the fixative, there was no such effect on the NADPH-diaphorase staining of olfactory nerves and glomeruli. Glomerular and olfactory nerve axons were not labeled with NPY antiserum.

These observations are consistent with previous reports that NADPH-diaphorase and APP (NPY)-like immunoreactivity are localized in the same cell populations of cortex and striatum. The selective staining of glomeruli may be an important functional marker of either the locus or the developmental state of the olfactory receptor cells which innervate these glomeruli and thus could be useful in functional studies of the olfactory bulb.

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286.12 SPECIES DIFFERENCES IN EXPRESSION OF SUBSTANCE P AND TYROSINE HYDROXYLASE IMMUNOREACTIVITY IN OLFACTORY BULB. H. Baker, Laboratory of Neurobiology, Cornell Univ. Med. Coll. New York, NY 10021.

Perikarya containing substance P-like (SP) immunoreactivity have been localized to the juxtaglomerular region of the hamster main olfactory bulb (Davis and Macrides, J. Comp. Neurol, 214:244, 1983). However, similar neurons have not been observed in either the mouse or rat main olfactory bulb. Utilizing a newly prepared high titer antibody to SP (Generously provided by Dr. R. Kream), the immunoreactive elements in rat, mouse and hamster olfactory bulb were investigated. In addition the distribution of SP immunoreactive cells and processes was compared with that of tyrosine hydroxylase (TH). The main olfactory bulb (MOB) of the hamster contained large numbers of both SP and TH positive juxtaglomerular neurons. TH, but not SP, containing neurons were also numerous within the external plexiform layer. For both antigens terminal arborizations were observed within the glomeruli and internal granule cell layer of the MOB. The hamster accessory olfactory bulb (AOB) also stained for SP with fibers and/or terminals within the mitral, internal plexiform and granule cell layers, and light processes within the glomerular layer. A few small, lightly-stained cells were localized to the granule cell layer. By contrast, SP staining in the rat MOB was limited to fine varicose fibers within the olfactory nerve and glomerular layers with no juxtaglomerular cell body staining. Numerous TH immunoreactive cells were predominant within the juxtaglomerular region and in comparison to the hamster few TH cells were found in the external plexiform layer. As opposed to the hamster AOB, where few SP cells were observed, the granule cell layer of the rat AOB contained numerous darkly stained SP positive cells. The fibers from these cells appeared to send axons through the inner plexiform layer to the mitral cell layer which contained numerous processes and the glomerular layer where only a few processes were found. The pattern of staining in mouse differed from both rat and hamster with no SP cell staining in either the MOB or AOB. Only fiber staining was observed. It was most prominent within the granule and mitral cell layers of the AOB. The TH staining resembled that observed in the rat with cells primarily in a juxtaglomerular position and processes filling the glomeruli. The mechanisms underlying the species differences in perikaryal staining for SP are not known. It is possible that the rat and mouse MOB juxtaglomerular neurons simply do not contain SP or that the peptide is present at levels below those detectable by the immunocytochemical procedures employed. Alternatively, SP may be present only in terminals of the rat and mouse MOB or the hamster and mouse AOB while the cell bodies contain a precursor which undergoes processing in terminals. These studies demonstrate that tyrosine hydroxylase is similarly expressed in different species while the expression of substance P is species dependent in neurons of the main and accessory olfactory bulb. (Supported by NSF grant BNS-8317552.)

## REGENERATION I

287.1 ENHANCEMENT OF ELECTRICAL COUPLING BETWEEN REGENERATING NEURONS BY STRESS. A.G.M. Bulloch. Dept. Physiol., Univ. Calgary, Alta., Canada T2N 4N1.

Adult neurons can exhibit sprouting in a number of situations not involving direct damage. The physiological consequences of such sprouting, however, are largely unknown. The identified neurons of the adult mollusc Helisoma have been utilized for studies of adult neuronal plasticity. For example, a new electrical synapse forms between the buccal neurons L5 and R5 when axonal regeneration is evoked by axotomy. Recently, specific forms of stress were found to evoke sprouting and retraction of neurites from neurons L5 and R5 in the intact nervous system. The present study examined the efficacy of the neuron 5-5 synapse in ganglia from previously stressed animals.

The experimental basis of this study was as follows. Animal were exposed to a stress (bleeding) known to cause sprouting of intact neurons 5. After 3 days, i.e., when the number of neurons 5 sprouted is maximal, ganglia were excised from both stressed and control animals and placed in organ culture in which axonal regeneration and neuron 5-5 electrical synapse formation occurs. Subsequent to physiological measurements each neuron was stained with Lucifer Yellow CH for assessment of sprouting.

The strength of the neuron 5-5 electrical synapse was substantially increased in ganglia from previously stressed animals. Specifically, the coupling coefficient between the neurons 5 after 2 days of culture was increased by 221% in experimental ganglia. This was due to a substantial reduction of coupling resistance (68%) and was in spite of a small decrease (26%) of non-junctional resistance.

A correlation between the degree of central sprouting and the strength of neuron 5-5 coupling has been observed in a number of previous studies. In this study, however, neurons sprouted to the same extent in both experimental and control animals. It is therefore possible that the increased electrical coupling could reflect more subtle metabolic changes such as increased synthesis of gap junction proteins.

It is concluded that stress can enhance the ability of neurons to form electrical synapses upon axonal regeneration. The modification of neuronal properties in stress could be important for a number of aspects of adult neuronal plasticity.

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287.2 A STRUCTURAL AND FUNCTIONAL COMPARISON OF NERVE RECONNECTION. J. Kerns, J. Siuciak* and H. Weinrib* (SPON: R. Penn). Depts. of Anatomy and Microsurgery, Rush Medical College, Chicago, IL 60304.

In the evaluation of new techniques for nerve repair, it is important to consider both structural and functional parameters. Rats were prepared for (a) sciatic nerve crush injury, (b) transection and reconnection by the method of de Medinaceli (Exp. Neurol. 81:469, 1983), and (c) conventional anastomosis with two 10-0 sutures. We have developed four quantitative methods to compare the nerve regeneration for the above three groups. (1) The "SCIATIC FUNCTIONAL INDEX" or SFI during the recovery period is determined by behavioral track analysis (de Medinaceli et al., Exp. Neurol. 77:634, 1982). The deficit values at one month are -13%, -79% and -88% for the respective lesion groups. (2) TOE-TWITCH TENSION recordings give functional data from the motor units. The deficits of the amplitude/area on the experimental side are -6/17%, -39/25% and -79/75% respectively. (3) NERVE MORPHOMETRY from transverse light microscopic sections and Bioquant computerized image analysis gives an estimate of the size and number of regenerated nerve fibers in the distal nerve segment. The change in the nerve fiber number is zero, +18% and +24%, while the reduction in fiber diameter is -41%, -48% and -51% respectively. The fiber density is variable, but the g-ratio remains fairly constant. (4) HORSERADISH PEROXIDASE (HRP) data is obtained by the retrograde transport from the distal segment past the lesion to the cell bodies. The mean nubmer of labeled motoneurons on the transection side at two months postoperative equals -31% compared to the control side. The cell body size and label intensity is not significantly different. The results suggest that following nerve transection and conventional repair, it is the reduction in the number of the motoneurons by 1/3 and the size of the distal fibers by 1/2 which best explains the 75-88% loss of function. It is not yet clear what is the role of the increase in the number of distal fibers by 1/4. We have confirmed independently that the reconnection method of de Medinaceli gives functional improvement over the conventional method of repair, but not as good as following a crush lesion. The structural data suggest an intermediate result as well, but less conclusively. (Supported by Grant NS 19769)

287.3 FUNCTIONAL RECONNECTION OF LOCOMOTOR CIRCUITRY IN THE SPINAL TRANSECTED SEA LAMPREY. A. H. Cohen, S.A. Mackler* and M.E. Selzer. Section of Neuroscience and Behavior, Cornell U., Ithaca, N.Y. 14853 and Depts. Neurology and Anatomy, U. of Pa. Sch. Medicine, Phila., PA 19104.

Large larval sea lampreys recover behaviorally from spinal transection. Their spinal axons are known to regenerate (Yin and Selzer, J. Neurosci., 3:1135-1145, 1983) and to form physiologically functioning synapses with target neurons distal to the transection scar (Mackler and Selzer, this Meeting). In order to determine whether synaptic regeneration is responsible for behavioral recovery the isolated spinal cords of previously transected lamprey larvae (Petromyzon marinus) 4-5 years old were bathed in physiological solution with 1 mM D-glutamate to activate "fictive locomotion" (Cohen and Wallen, Exp. Brain Res., 41:11-18, 1980). The motor discharges were recorded simultaneously from ventral roots above and below the healed transection.

Two types of fictive locomotor patterns were observed in untransected control larvae: 1) slowly propagating waves with burst durations of 2.0-5.0 sec. and phase lags of approximately 3% of the cycle per spinal segment; 2) rapidly propagating waves with burst durations of 0.3-2.0 sec. and phase lags of less than 1% of the cycle per segment. All eight animals which had recovered at least two months from spinal transection at mid-body showed some degree of recovery of intersegmental coordination during fictive locomotion. Seven of 8 recovered coordination of fast swimming. Three animals recovered coordination of slow simming. Median values for phase lags between segments on opposite sides of the transection scar were in the normal range for all but one fast and one slow swimming pattern. Retransection eliminated the coupling so that phase lags between reseparated segments became randomly distributed. Therefore, synaptic regeneration in lamprey spinal cord includes components of the circuits for intersegmental coordination of locomotor pattern generation. Supported by NIH grants NS16803 and NS14837 and by the American Paralysis Association.

287.4 ELEMENTS OF SPECIFICITY IN SYNAPTIC REGENERATION BETWEEN LAMPREY SPINAL NEURONS. S.A. Mackler* and M.E. Selzer. Depts. of Anatomy and Neurology, U. of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Spinal axons of 4-5 year-old larval sea lampreys regenerate following spinal transection. We now report that neurons above and below the transection scar can reestablish monosynaptic connections with each other in a way which shows similarities with the original pattern of interconnections.

In control animals intracellular stimulation of giant interneurons (GIs) elicited composite electro-chemical EPSPs in 50% of other GIs located rostrally. These followed stimulation at 3-33 Hz with fixed latencies and persisted in 20 mM $Ca^{++}$ Ringer's. In animals recovered 6 weeks or more from spinal transection, stimulation of GIs located below the scar elicited EPSPs in 7 of 35 GIs (20%) located within 8 mm above the scar. Since it is probable that 50% of the GI axons regenerated (Yin and Selzer, J. Neurosci. 3:1135-1144, 1983), the proportion of regenerated axons which formed synapses with rostral GIs was approximately 40%. As in control GI-GI connections, the regenerated ones were monosynaptic composite electro-chemical EPSPs. However, the chemical components in regenerated synapses were significantly larger than those of controls (2.4 mV $\pm$ 0.6 SEM vs. 0.7 mV $\pm$ 0.1) and the electrical components were smaller (0.9 mV $\pm$ 0.1 vs 1.5 mV $\pm$ 0.2).

In control animals no dorsal cells (DCs) were monosynaptically linked to rostral GIs in 19 cases. In transected animals none of 42 DC-GI pairs were synaptically linked across the scar. Similarly none of 14 giant reticulospinal axons were synaptically linked to GIs below the scar, also in conformity to the normal pattern.

Finally, DCs (intraspinal primary sensory cells) normally receive no synaptic input. In animals recovered from spinal transection whole cord stimulation, either across the scar or on the same side, failed to elicit synaptic activity in any of 55 DCs.

We conclude that: 1) Regenerating axons of lamprey GIs can form functioning monosynaptic connections with other GIs distal to the scar. 2) The frequency and characteristics of such connections are similar to those in the normal spinal cord. 3) The axons of two types of cells which normally do not contact GIs fail to do so after regeneration and one type of cell which ordinarily receives no synaptic input remains so. (Supported by NIH grant NS14837 and by the American Paralysis Association).

287.5 EVIDENCE FOR REGENERATION OF SPECIFIC SYNAPTIC CONNECTIONS IN APLYSIA FOLLOWING CNS LESIONS. S.M. Fredman. School of Biological Sciences, University of Kentucky, Lexington, KY 40506

The mollusc Aplysia has been used to investigate the cellular mechanisms of behavioral plasticity. Behavioral, morphological and physiological evidence has now been obtained that demonstrates that Aplysia exhibits another form of plasticity: Regeneration and recovery of function.

An electric shock to the tail of Aplysia evoked a stereotyped escape locomotor sequence. This response was selectively abolished by crushing both cerebro-pleural (C-PL) connectives. The effects of the lesion were specific. Spontaneous and food-evoked locomotion was unimpaired. Prior to lesioning, the mean latency of the response was 13.6 $\pm$ 3.1 sec (5 subjects, 45 trials). Two days after lesioning, escape locomotion was absent (latency > 60 sec), although tail withdrawal and inking could still be evoked. Behavioral recovery was gradual. Partial responses at long latency were observed 7-14 days postlesion. By 24 days postlesion, recovery was complete; lesioned animals again exhibited the full escape locomotor response. The latency (16.5 $\pm$ 4.7 sec) was not significantly different (t-test, $p = 0.01$) from prelesion values.

Behavioral recovery was paralleled by both morphological and physiological changes in lesioned neurons. This was studied in the isolated nervous system. Intracellular injections of cerebral ganglion neurons with nickel-lysine (Fredman, in preparation) revealed the sprouting of new axon-like processes as well as smaller diameter neurites. Axon-like sprouting occured from or near the cell body not from the stump of the lesioned axon. By the time behavioral recovery was complete, it was possible to demonstrate that a neural correlate of the escape locomotor response triggered by tail shock also had recovered. Electrical stimulation of the posterior pedal nerves normally evokes IPSPs in cerebral B neurons which are mediated by pleural interneurons. Synaptic input evoked by posterior pedal nerve stimulation was absent both immediately after crushing the C-PL connectives and prior to the initial phase of behavioral recovery. This input returned 14-20 days postlesion. Finally, it was possible to demonstrate that specific excitatory and inhibitory chemical monosynaptic connections between cerebral B neurons and identified pleural neurons were re-established.

From this it is concluded that Aplysia is capable of axonal regeneration which can restore specific synaptic connections and behavioral function.

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287.6 STRUCTURAL AND FUNCTIONAL ASSESSMENT OF THE REINNERVATION PATTERN OF CAT EXTRAOCULAR MUSCLE FOLLOWING CENTRAL CUT OF THE IIIrd, IVth AND VIth CRANIAL NERVES. R. Baker, C. Peck, R. F. Spencer, J. Delgado-Garcia* and J. Winterkorn. Dept. Physiol. & Biophys., New York Univ. Med. Ctr., New York, N.Y. 10016.

Within 2-3 weeks following peripheral cut of any extraocular nerve near its muscle insertion site, reinnervation occurs and normal oculomotor function returns. Delgado-Garcia et al. (1981) found subtle changes in motoneuronal properties, afferent organization and muscle fiber composition accompanying axotomy, but they were reversible upon reinnervation. We have studied eye movements, motoneuron physiology and morphology including organization of the extraocular nuclei and muscles in 8 cats 1-2 years following either mono- (3) or binocular (5) central cut of nerves III, IV and VI near the cavernous sinus at 4-6 weeks of age. Reinnervation occurred within 2-4 months as indicated largely by the return of retraction rather than rotational eye movements. Measurement with a magnetic coil system revealed that eye movements were not conjugate, of small amplitude (gains $\leq$0.1 of normal) and frequently in directions opposite to those expected in response to visual or vestibular stimuli. Resistance to mechanical displacement of the globe was high, yet dissection of the orbital contents revealed no adhesion or other abnormality underlying the stiffness. Reinnervated muscles appeared normal by light and electron microscopy. Since these data suggested that the pattern of motor reinnervation might be altered we injected HRP into either all (3 cats) or individual (3 cats) extraocular muscles. To our surprise we found the oculomotor nuclei subgroups degenerated in all 6 cats suggesting reinnervation of extraocular muscles by the quantitatively fewer (never more than 50%) motoneurons in the trochlear and abducens nucleus. Isolated injection of either medial or inferior rectus muscles confirmed innervation by motoneurons from both IV & VI. We conclude that the abnormal eye movements are in part explained by this ubiquitous IV & VI reinnervation scheme. The remarkably peculiar resiliency of some abducens motoneurons to degeneration was also used to examine Sperry's re-specification hypothesis. In two cats, we recorded from antidromically identified abducens motoneurons that largely reinnervated the medial rectus muscle as inferred by stimulation of the VIth nerve and eye movements. Direction, position and velocity sensitivity of the motoneurons were unchanged, leading to the conclusion that there was no central adaptive readjustment to re-specify oculomotor activity. In general, we surmise that the role for a particular motoneuron is specified early in ontogeny and is independent of the muscle innervated as well as being impervious to sensory reafference from inappropriate motor responses.

287.7 ALTERED PATTERN OF SKELETAL MUSCLE REGENERATION AFTER X-IRRADIATION. A.K. Gulati* (SPON: H.G. Goshgarian). *Department of Anatomy, Medical College of Georgia, Augusta, GA 30912.*

*Devascularization injury to adult skeletal muscle caused by experimental autotransplantation produces degeneration of majority of original myofibers. Upon revascularization, the surviving precursor myosatellite cells become activated, proliferate, and fuse to form new myotubes and myofibers. This study describes alterations in specific events involved in myofiber degeneration and regeneration due to X-irradiation. Rat extensor digitorum longus muscle was removed, irradiated to administer 2,000 or 10,000 Rads (Clinac 4 x-ray machine; 750 R/min), and transplanted back into the same animal. Muscle transplants from unirradiated controls and each irradiated group were analyzed between 4 and 28 days at light and electron microscopic level. Muscles in the control group underwent rapid degeneration and regeneration and by 14 days were filled with regenerated myotubes and myofibers. Muscles exposed to 2,000 Rads underwent similar degeneration and myosatellite cell activation; however, no fusion of cells to form myotubes was evident. In muscles exposed to 10,000 Rads, myofiber degeneration and myosatellite proliferation was not as rapid but was clearly evident. Again no fusion of myosatellite cells was seen. After 14 days muscle from both irradiated groups appeared similar and resembled dense connective tissue with many fibroblasts, macrophages and thick collagen bundles. Many isolated (un-fused) myoblasts were also present in these later grafts, they remained undifferentiated and did not express any myofilaments and lacked basal lamina. These results reveal that inital events involved in muscle regeneration such as revascularization, myofiber degeneration, myosatellite cell activation and proliferation appear not to be altered by irradiation. However, later events such as myoblast differentiation, their fusion to form myotubes, formation of contractile myofilaments and basement membrane are inhibited. The mechanism by which irradiation affects these processes is currently unknown, but it may be that irradiation, in part, produces alterations in the cell surface components important for myoblast differentiation, fusion and subsequent maturational events.*

287.8 SPECIFIC RESTORATION OF HIPPOCAMPAL NERVE CONNECTIONS BY DENTATE GRANULE CELLS TRANSPLANTED TO X-IRRADIATED RATS. J. Zimmer*, N. Sunde* and T. Sørensen* (SPON: I. Divac). Inst. of Anatomy B (Neurobiology), University of Aarhus, Aarhus, Denmark.

The rat dentate gyrus is a useful model for repair of specific nerve connections by intracerebral transplants: - 1) The major afferents, the two entorhinal perforant pathways (PP) and the hippocampal commissural-associational system, are segregated in laminae along the granule cell dendrites, and the efferents, the mossy fibers (MF), terminate in a well-defined layer along the hippocampal CA3 pyramidal cells. - 2) Most granule cells develop after birth. Their formation can therefore be stopped by neonatal X-irradiation, leaving the irradiated rats to grow up with only 10-20% of the granule cells, a corresponding small MF projection and aberrant PP projections to CA3 (Laurberg and Hjorth-Simonsen, *Nature, 269*: 158, 1980). The late development moreover makes the cells easily available as transplantable, well-surviving neurons.

Taking advantage of these features we attempted to replace the damaged granule cells by transplants of intact cells from newborn donors, either immediately after irradiation (600 rad bilaterally at birth) or with a delay of up to several months. The recipient brains were processed and examined six weeks or more after transplantation, using Timm staining for transplant MF and host PP connections, silver staining and electron microscopy for host commissural and PP connections and AChE staining for cholinergic host septohippocampal fibers.

Transplantation within 3 weeks after irradiation, i.e. within the normal developmental period of the recipients, revealed a remarkable capacity of the granule cells to exchange specific nerve connections with the host, provided the transplant was properly located. Host commissural and PP fibers thus terminated in appropriately sized and spaced laminae in the transplant dentate molecular layer, and transplant MF normalized the MF-zone in the host CA3 (Sunde et al., *Nature, 310*: 51, 1984). With further delay of transplantation the exchange of host-transplant connections decreased. Host commissural afferents to the transplants vanished, and host PP became confined to thin laminae superficial in the transplant molecular layer. Also the transplant MF projection to the host weakened. Only the AChE-stained, cholinergic host projection to the transplants appeared unaffected by the delay and increase in recipient age.

The present observations have interesting reparative implications, but we also want to emphasize the developmental aspects. The mutual changes of terminal territories in the transplant molecular layer seen with decreasing host PP ingrowth thus suggests that the laminar segregation of the afferents primarily is determined by interaxonal competition and not axodendritic specificity.

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287.9 NEOCORTICAL TRANSPLANTS IN THE SI CORTEX OF ADULT MICE RECEIVE THALAMIC AFFERENTS FROM THE POSTEROMEDIAL (POm) BUT NOT THE VENTROBASAL (VB) NUCLEUS. D.T. Ross* and F.F. Ebner, Center for Neural Science, Brown University, Providence RI, 02912.

The primary somatosensory cortex (SI) of adult mice receives convergent input from several thalmic nuclei including the ventrobasal complex (VB) and posteromedial nucleus (POm). In the present study embryonic neocortical tissue was transplanted into the SI cortex of adult BALB/c mice and the ingrowth of thalamocortical axons from the VB and POm was examined using anterogradely transported HRP.

Neocortical tissue from embryos in their 12th to 19th gestational days was transplanted into the somatosensory cortex of adult BALB/c mice. Two to six months later HRP (0.05ul of 25% Sigma type VI) was injected at the coordinates of the VB or POm. 18-24 hours later the animals were sacrificed and their brains processed for the histochemical visualization of HRP using TMB as the chromagen (Adams, 1980).

No thalamic afferent ingrowth to the transplants was seen in any of the cases which received HRP injections restricted to the VB. Despite the presence of HRP terminal labeling in layer IV immediately adjacent to the transplant and the presence of retrogradely labeled pyramidal neurons in layer VI ventral to some transplants, axons labeled by injection of HRP into the VB were never found to cross the host brain/transplant interface. Injections of HRP into the POm did result in the labeling of a meager thalamic afferent projection to the transplants. Anterogradely labeled axons were found to enter the transplants from the host white matter and layer V and to arborize locally within the transplant parenchyma.

The differential ability of the two thalamic nuclei to innervate the transplants appears to reflect the differential sensitivity of VB and POm neurons to axotomy. Cortical lesions made by transplantation into the SI cortex produced retrograde degeneration in topographically corresponding regions of the VB, as seen in Nissl stained sections and sections reacted for GFAP immunohistochemistry. Neuronal degeneration and the accompanying reactive astrogliosis occur in the POm only following extensive ablation of the somatosensory motor cortex. Focal cortical lesions produced by transplantation into the SI cortex appear to extensively axotomize the VB neurons that project densely to narrow regions of SI but only partially axotomize the POm neurons that project widely and terminate diffusely over several cortical areas. The "sustaining" collaterals of the POm neurons in undamaged regions of the cortex may both protect the nonspecific thalamocortical neurons from retrograde degeneration and allow them to express a capacity for axonal regeneration. (Supported by NIH grant NS13031-09).

287.10 EPENDYMAL CELLS IN HOMOGRAFTS OF RAT E14 FETAL CORTEX TRANSPLANTED TO ADULT RAT SPINAL CORD INDICATE THE ORIGIN AND EPICENTERS OF TRANSPLANT GROWTH. J. J. Bernstein and Yipeng Tang* Lab. CNS Inj. and Regen., V.A. Med. Ctr., Wash., D.C. 20422 and Depts.of Neurosurg. and Physiol., George Washington. Sch. Med., Wash., D.C.

The dividing ventricular layer of fetal cortex and spinal cord differentiates into ependyma approximately at birth or shortly thereafter. Rat cortical ependyma are cuboidal or squamous in shape whereas spinal ependyma are columnar or psuedo-columnar. Cortical and spinal ependymal cells are ciliated and posses villi at the luminal surface. Thirty-eight Sprague Dawley male rats (350g) had E14 fetal rat cotex (0.5X1.0 mm,minced pieces) pressure injected (30g needle on 50ul Hamilton syringe) between the dorsal horn and dorsal column of the spinal cord under the T6 vertebra (N=5, at 7,14,21,30,45,60,90 DPO,and 3 normals). The tissue was prepared for light and electron microscopy. At 7 and 14 DPO there are actively dividing layers of cells. Up to 60 days after transplantation dividing cells differentiate into ependyma (based on cytoplasmic constituents) in the parenchyma of the transplant. By 21 DPO cysts are observed in the transplanted spinal cord which are lined by columnar and psuedocolumnar ependymal cells (derived from the host), cuboidal and squamous ependymal cells at the base of the cortical homograft (derived from the transplant) and neuroglia. Psuedomorphic reactive ependymal cells are also present with cilia and villi on one surface. Ependyma form cilia and villi lined channels or spaces in a myriad of combinations and are joined by tight junctions when contiguous. Within the body of the transplant rosettes are formed by dividing cells, ependymal and subependymal cells. These data show that although the cortical homograft is in spinal cord, which alters the phenotype, cortical ependyma differentiates, indicating the preservation of transplant genotype. In addition, there are multiple epicenters for growth in the transplant as indicated by the location and morphology of ependymal cells. Supported by the Veterans Administration. Dr. Tang is a visiting scholar: Central Laboratory, Beijing Coll. Trad. Chinese. Med., Beijing, Peoples Republic of China.

287.11 AChE-POSITIVE AXONAL SPROUTING AND REGENERATION ACROSS SCAR TISSUE IN ADULT RAT BRAIN. J.S. Wendt* and K.A. Ayyad* (SPON:H. Feit). Dept. of Neurology, VA Medical Center and iv Unof Tex Health Science Center, Dallas TX 75216.

After hippocampal fimbria transection in the adult rat we have noted that acetylcholinesterase (AChE)-positive fibers (1) have a limited capacity for sprouting and growth over cellular substrata, (2) occasionally grow across a post-traumatic tissue bridge between the septum and caudate nucleus, and (3) can exhibit plexus-like growth in regions of marginal injury. To investigate further the regenerative potential of AChE-positive fibers, the following study was conducted: a 2mm-wide, 3mm-deep incision was made in the right sensorimotor cortex in the coronal plane 1.3mm posterior to the bregma suture line. After survival periods of 0,3 and 6 hours; 1,2,3,4 and 10 days; 2,3 and 4 weeks; and 2 months; the animals were sacrificed, and frozen horizontal brain sections were stained for either AChE (cresyl violet counterstain) or astrocytes by Cajal's gold chloride method.

At 0 hr the brain cytoarchitecture around the lesion was preserved. At 3 hr axonal swelling and a suggestion of growth cone formation was noted at the rostral margin of the lesion. Neurite extension from growth cones was seen at 6 hr. Over time there was further neurite outgrowth, accumulation of AChE stain at the rostral margin of the lesion, and retrograde extension of axonal swelling. By day 7 the rostral and caudal margins of the lesion were apposed, and extensive AChE-positive fiber growth was seen through the scar. Numerous fibers entered the scar rostrally, and many fibers extended from the scar into caudal brain tissue. Progressive reaccumulation of AChE-positive fibers caudal to the lesion occurred up to the 2-month endpoint. Astroglial reaction was evident at day 3, was maximally developed at 2-4 weeks, and was diminished by 2 months.

The results indicate that AChE-positive axons are capable of sprouting and regrowth through scar tissue after trauma in adult rat brain.

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287.12 REGENERATED VISUOTECTAL PROJECTIONS OF HETEROTOPIC GRAFT CHIMERIC EYES OF XENOPUS LAEVIS. R. Tompkins, M. Miller*, B. Bartholomew*, B. Szaro and D. Reinschmidt*. Dept. of Biology, Tulane Univ., New Orleans, LA 70118.

Heterotopic grafts of portions of stage 32 eyebuds of Xenopus laevis embryos may develop visuotectal projections which reflect the embryologically determined tectal affinities of the graft. Such mosaic projections have tectal responses that are stimulated by two different areas of the visual field. However, identical grafts may instead develop normal visuotectal projections despite the continued presence of graft-derived retinal ganglion cells in the chimeric eyes. Such regulated projections have been explained in terms of signalling from the host retinal surround. The tectal affinities of retinal ganglion cells of mosaic and regulated chimerae were assessed by visuotectal mapping following intraorbital optic nerve crush and prolonged regeneration.

Pigment and ploidy marked ventral into dorsal heterotopic grafts were performed at stage 32 and the visuotectal maps of the chimeric eyes assessed 3-6 weeks after metamorphosis. Unoperated control eyes with normal visuotectal projections at that age required 3 to 6 months to regenerate normal projections. Therefore, mapping of regenerated visuotectal projections of chimeric eyes was done after 6 months of regeneration. Heterotopically grafted chimeric eyes with mosaic projections after metamorphosis regenerate mosaic projections. Those with initially regulated visuotectal projections yielded two visuotectal projection patterns following regeneration. Eleven of 16 animals regenerated mosaic maps. This result was correlated with the presence of graft-derived retinal ganglion cells in the chimeric eye. The duplicated visual fields occupied that portion of visual space viewed by the graft-derived cells and elicited responses in those portions of the tectum predicted by the position of origin of the grafts. Five of the 16 animals regenerated regulated projections but this result was correlated with the absence of retinal ganglion cells derived from the graft in these eyes. These results suggest that heterotopic grafts which develop normal visuotectal projections do not do so because of changes in the embryologically determined tectal affinities of the retinal ganglion cells. Rather, these results suggest that the dynamics of eye growth and fasciculation of the retinal ganglion cell axons can subvert the determination of the grafted cells. Signalling resulting in changes in positional information need not by invoked to explain regulated patterns. Indeed, such changes in determination are incompatible with the regeneration of mosaic projections from initially regulated projections.
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## SPECIFICITY OF SYNAPTIC CONNECTIONS II

288.1 SPECIFIC REGENERATION OF DEVELOPING SPINAL MOTONEURONS IN THE ABSENCE OF PERIPHERAL SENSORY FIBERS. Paul B. Farel and Sibyl E. Bemelmans*. Dept. of Physiology, Univ. N. Carolina Sch. of Medicine, Chapel Hill, NC 27514.

Horseradish peroxidase (HRP) applied to circumscribed hindlimb regions of the bullfrog tadpole (Rana catesbeiana) retrogradely labels motoneurons located in particular regions of the lumbar lateral motor column (LMC). The localization of retrogradely labeled motoneurons has been used to assess the specificity of neuromuscular connectivity during early development (J. Comp. Neurol., in press) and after regeneration of motor axons following ventral-root transection (Soc. Neurosci. Abstr., 9:373, 1983).

At early stages of development, retrogradely labeled motoneurons are as localized along the dorsal-ventral and rostral-caudal axes of the LMC as they are in juvenile frogs. Following ventral rhizotomy before st. VI, retrogradely labeled motoneurons are as localized as in unoperated tadpoles. When ventral roots were transected between st. VI and VIII, localization following regeneration is present along the dorsal-ventral axis but is lost along the rostral-caudal axis. Localization of retrogradely labeled motoneurons is largely lost in tadpoles operated after st. IX. We are interested in the cues which guide regenerating axons to the appropriate limb regions at early developmental stages.

Sensory axons, particularly muscle afferents, could serve as a source of guidance information. For example, muscle spindle afferents, which normally make monosynaptic contact with the motoneurons supplying that muscle, may provide cues that can be read by motor axon along their regenerative course. To test this possibility, the dorsal and ventral roots were transected and the dorsal root ganglion removed in the three spinal segments that provide innervation to the hindlimb. Despite the fact that this operation left a 2-3 mm gap between the proximal stump of the ventral root and the distal stump of the spinal nerve, the hindlimb was usually reinnervated during the 6-8 week postoperative survival period. Following application of HRP to circumscribed regions of the hindlimb, retrogradely labeled motoneurons were as localized in the LMC as in tadpoles that had undergone ventral-root transection without damage to sensory fibers. At st. V, localization along both the dorsal-ventral and rostral-caudal axes of the LMC was found. Between st. V and VIII, localization was present along the dorsal-ventral axis, but was not evident along the rostral-caudal axis. Thus, neither peripheral nor central branches of sensory fibers appear to be necessary for motoneurons to regenerate to appropriate limb regions.

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288.2 THE SOMITIC ORIGIN OF LIMB MUSCLES IN THE CHICK EMBRYO: A CORRELATION WITH MOTOR INNERVATION. C. Lance-Jones. Dept. of Anatomy and Cell Biology, Sch. of Med. and Ctr. for Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA 15261.

There is good evidence in the chick embryo that limb innervating motoneurons show a considerable selectivity for paths or targets within the limb, yet the developmental basis for this selectivity is not known. Studies of the reinnervation of transplanted mammalian trunk muscles suggest that target selectivity may be based on a positional quality shared by neurons and their targets (Wigston, D.J. and Sanes, J.R., Nature, 299:464, 1982). As a first step in addressing this hypothesis in the chick limb system, I have examined the relationship between the somitic level of origin of myogenic cells within limb targets and their segmental innervation pattern. One to two identified lumbosacral (LS) somites were transplanted from stage 15-17 quail embryos to an equivalent position in similarly staged chick embryos. Some overlying ectoderm was frequently included in the transplant, however its presence or absence did not appear to affect results. Chimeras were sacrificed at stage 27-31. Serial sections were stained by the Feulgen method and examined for the presence of quail cells within individual limb muscles.

Myogenic cells arising from a specific quail somite or pair of somites populated a discrete subset of limb muscles. For example, following transplantation of anterior somites such as LS 1 or 2, quail cells were found only in anterior thigh and pelvic muscles; following transplantation of posterior segments such as LS 5 and 6, quail cells were present only in posterior thigh and shank muscles. The pattern of somitic contribution to an individual chick muscle appeared to relate to its known segmental innervation pattern. For example, the sartorius, a muscle normally innervated in the chick by spinal cord segments LS 1 and 2, contained quail cells only when somites LS 1 or 2 were transplanted. While the specific somitic origin of all limb muscles has yet to be determined, in all embryos examined (n=13) transplanted somites only contributed to muscles or muscle regions normally innervated by cord segments adjacent to the transplant region.

These data indicate a correlation between the axial level of origin of motoneurons and the myogenic cells of their target. Somite manipulations are presently being carried out to determine whether target or pathway choice might be based on these shared positional characteristics.

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288.3 SELECTIVE REINNERVATION OF AXOLOTL LIMB MUSCLES. D. J. Wigston. Physiology Department, Emory University, Atlanta, GA 30322.

Mammalian intercostal muscles transplanted from different thoracic segments to the neck are selectively reinnervated by subsets of the autonomic preganglionic axons in the cervical sympathetic trunk that originate from appropriate rostro-caudal levels of the spinal cord (Wigston and Sanes, Nature 299:464, 1982; J. Neurosci. 5:1207, 1985). Previous work on the regeneration of motor nerves to mammalian limb muscles, however, indicated a lack of specific reinnervation. The difference between these results suggests two possibilities: 1) regenerating motor axons may lack the selectivity expressed by preganglionic axons, and 2) individual limb muscles, which each develop from several somites, may lack the unique recognition labels that segmented intercostal muscles seem to have. I am presently examining this further by studying the reinnervation of axolotl hindlimb muscles.

To test if limb muscles can be selectively reinnervated by motoneurons I transplanted individual muscles, whose segmental innervation I had determined in other experiments, to new positions in the limb and studied the segmental origin of the axons that reinnervated them. This approach provides a more direct test of the existence of target recognition cues than limb denervation or rotation experiments which require axons to regenerate over long distances to their original muscles. I removed two adjacent knee extensors, the anterior and posterior iliotibialis (ILT), which have distinct segmental innervation patterns, and exchanged them so that the original anterior ILT occupied the site normally inhabited by the posterior ILT, while the posterior ILT assumed the position vacated by the anterior ILT. Intracellular recording 6-13 weeks later showed that both muscles were reinnervated according to their initial rather than their new position. The innervation of transplanted anterior ILT muscles was consistently of more rostral origin than that of transplanted posterior ILT muscles, and closely resembled that of control anterior ILT muscles removed and reimplanted in their original position.

These results show that 1) regenerating motor axons, as well as preganglionic axons, are capable of selective synapse formation; and 2) different limb muscles, like intercostals, can be distinguished from one another by regenerating axons. The existence of a mechanism for selective synapse formation that can contribute significantly to the restoration of specific nerve-muscle connections suggests that the reason transected limb nerves fail to re-establish specific connections in adult mammals is that regenerating axons fail to reach the vicinity of their original muscle and therefore do not reveal their target preference. (Supported by NIH grant NS20965.)

288.4 THE EFFECT OF BLOCKING FUNCTIONAL ACTIVITY AND MOTONEURON CELL DEATH ON THE ACTIVATION PATTERNS OF CHICK LUMBOSACRAL MOTONEURONS. L. Landmesser, M. Szente*, and L. Dahm. Dept. of Physiology and Neurobiology. Univ. of CT, Storrs, CT 06268.

Chronically applied neuromuscular blocking agents have been shown to prevent normal motoneuron cell death during development of the chick lumbosacral spinal cord (Pittman & Oppenheim, J. Comp. Neurol. 187: 425). Since cell death has been implicated in refining neural circuits, we investigated the effect of daily in-ovo doses of dTC sufficient to block activity and cell death on the activation patterns of hindlimb motoneuron pools; these were assayed by EMG or muscle nerve recordings in isolated spinal cord-hindlimb preparations at st 36.

Chronically treated preparations, where motoneuron death was later shown to be completely blocked, were isolated and allowed to recover in Tyrodes. Although the neuromuscular junctions rapidly recovered and could be activated by direct spinal nerve stimulation, the cords were unable to produce the patterned bursts characteristic of control preparations for several hours. However, with sufficient washing (5-7 hrs) the cords recovered and were able to produce typical alternating sequences of flexor and extensor activity that differed only slightly from control cords. The blockade of patterned activity and the minor changes that persisted after washing appeared to be due to a direct effect of dTC on the spinal cord. This was confirmed by showing that similar effects were brought about by single in-ovo injections of dTC 10 hrs prior to sacrifice or by bath application of $1 \times 10^{-6}$ M dTC to isolated cords. We also found that bath-applied αBTX (3-4 μg/ml) in concentrations sufficient to block the neuromuscular junction also prevented patterned bursting in isolated cords.

We conclude that some cholinergic circuits within the cord that are necessary for patterned activation of motoneurons are blocked by in-ovo levels of dTC and αBTX required to block the neuromuscular junction. It is therefore possible that other similarly applied drugs may also have direct effects on the CNS. However, since cords which recovered from the drugs were able to produce normal activation patterns, we also conclude that neither motoneuron cell death nor the patterned activation of motoneurons is needed for the development of the basic neural circuits responsible for the normal alternating pattern of flexor and extensor motoneuron activation.

Supported by NIH grant NS 19640.

288.5 PHENOTYPE AND NEURAXIS POSITION OF FETAL MOUSE SPINAL CORD NEURONS DETERMINE THE AMOUNT OF CHOLINERGIC PROJECTIONS INTO CO-CULTURED SUPERIOR CERVICAL GANGLIA. A.Chalazonitis, S.M.Crain and J.A.Kessler#. Depts. of Neuroscience and Neurology#, Albert Einstein Coll. of Medicine, Yeshiva University, Bronx, N.Y. 10461

The specificity of CNS innervation of sympathetic ganglia was analyzed with organotypic explants of fetal mouse spinal cord (E13-14) and superior cervical ganglion (SCG;E17). Meninges-free cord from upper thoracic levels (C8-T4) was dissected in "open-book" orientation and cut longitudinally into dorsal (D), medial (M) and ventral (V) strips. To reduce surgical damage to the autonomic preganglionic neuron population in M cord, strips consisting of both M+D and M+V cord were prepared for comparison with isolated D and V strips. Unilateral strips were co-cultured with a single SCG; bilateral strips with a pair of SCGs.

Fascicles of neurites formed bridges across the gaps (0.5-1 mm) between the cord and SCG explants. After 4 wks of maturation in vitro, cholinergic projections into SCGs were quantitated by choline acetyltransferase (ChAT) assays of individual ganglia. ChAT activity (pmole product/ganglion/hr) was 98±12 (n=15) in SCGs co-cultured with MD cord, as compared to only 15±2 (n=24) in SCGs co-cultured with D cord and 10±1 (n=18) in SCGs grown alone. Similarly, SCGs co-cultured with MV cord contained higher ChAT activity (72±7; n=44) than those with V cord (35±5; n=18). Furthermore, co-culture of MV cord with an inappropriate target, lumbar dorsal root ganglion (DRG), elevated ChAT activity in this ganglion by < 18% of the value in similarly co-cultured SCGs.

Transverse sections of cord from different levels of the neuraxis were also co-cultured with pairs of SCGs. ChAT activity in SCGs co-cultured with T5 and T1/T2 cord was remarkably high: 317±87(n=6) and 176±18 (n=33), respectively, whereas SCGs with less appropriate levels of the cord, T9 and L2/L3, contained less enzyme activity:89±20 (n=10) and 18±4 (n=11). Electrophysiologic analyses and control tests are in progress to ascertain that the increased ChAT activity in co-cultured SCGs does, in fact, reflect innervation by specific cord neurites.

These data suggest: I) phenotypic preference for (a)'innervation' of SCGs by co-cultured cholinergic, presumably autonomic preganglionic, neurons located in medial cord, rather than by dorsal or ventral cord neurons; and (b) projections by cholinergic cord neurons into appropriate SCG targets rather than inappropriate DRG tissue; II) positional preference for 'innervation' of SCGs by appropriate cord neurons in upper thoracic levels. These conclusions are consistent with studies of specificity of sympathetic ganglion innervation by cord neurons in situ (Lichtman et al, J.Physiol.'80; Rando et al, J.Comp. Neurol.'81; Rubin, J.Neurosci.'85). Supported by NINCDS research grants 17572 (AC), 20778 & 20013 (JK).

288.6 SUBPOPULATIONS OF SYMPATHETIC NEURONES DIFFER IN THEIR REGENERATIVE CAPACITIES AND THEIR RESPONSES TO POSTNATAL REMOVAL OF NERVE GROWTH FACTOR. C.E. Hill*, I.A. Hendry*, M.C. Ngu* and D.F. van Helden*. (SPON: S.J. Redman). Dept. of Pharmacol., John Curtin Sch. of Med. Res., Canberra, A.C.T. Australia, 2601.

Rapid freezing of a mesenteric artery supplying the ileum of the rat leads to a sympathetic denervation of the artery distal to the point of freezing and of the enteric neurones within the segment of gut supplied by that artery (Furness, J.B., Histochemie, 21: 295, 1970). In the subsequent weeks after this operation, there is a more rapid return of sympathetic control of gut motility than of arterial contractility, the early regenerating fibres growing alongside the denervated artery to reach the enteric neurones. Since sympathetic denervation has been reported to result in an increase in nerve growth factor (NGF) in target tissues (Ebendal, T. et al., Nature, 286: 25, 1980), we have investigated whether the populations of sympathetic neurones innervating the mesenteric arteries and enteric neurones are differentially sensitive to NGF.

Neonatal rats were injected daily with antiserum to NGF (kindly supplied by Dr. R.A. Rush) or saline for the first postnatal week. Mesenteric arteries and segments of ileum were tested both physiologically and histochemically for the presence of a sympathetic innervation when the animals were 4 and 8 weeks old. At both ages, stimulation of the paravascular nerves in animals treated with anti-NGF led to a decrease in spontaneous gut motility, while a single perivascular stimulus failed to elicit the excitatory junction potential seen in control arterial preparations, nor did repetitive stimulation produce arterial constriction. Fluorescence histochemistry confirmed that the intramural ganglia were innervated by noradrenergic fibres in a manner similar to that of control animals while the mesenteric arteries of the same treated animals completely lacked a sympathetic innervation. Retrograde labelling of nerve cell bodies with the fluorescent dye, fast blue, in control and antiserum treated rats showed that the neurones innervating the intramural ganglia were situated in the prevertebral, coeliac and superior mesenteric ganglia and in ganglia along the greater splanchnic nerves (splanchnic ganglia). By inference from studies in control animals in which both populations of cells were labelled, the neurones supplying the mesenteric arteries were located in prevertebral and splanchnic ganglia and in the thoracic and lumbar paravertebral sympathetic chains.

We conclude that the sympathetic neurones innervating the mesenteric vasculature require NGF postnatally for their survival, while those innervating the enteric ganglia do not. The neurones comprising these two populations appear to overlap considerably in their distributions throughout the prevertebral and splanchnic ganglia although only neurones supplying the blood vessels are found in the paravertebral chains.

288.7 CLUSTERED SYNAPSE FORMATION BY EARLY REGENERATING RETINOTECTAL FIBERS PRECEDES THE SUBLAMINAR REDEPLOYMENT OF RETINAL CONNECTIONS. W.P. Hayes and R.L. Meyer. Developmental Biology Center, University of California, Irvine 92717.

After optic nerve crush in goldfish retinotopically appropriate tectal connections are regenerated in two phases. During the first 40 days gross retinotopography is reformed without impulse activity. The development of refined retinotopography over the next several months is activity-dependent(Meyer, 1983, Dev. Brain Res. 6:293). In this study the horseradish peroxidase(HRP) labeled retinotectal projection to the Optic Fiber(SO) and Superficial Fiber and Gray(SFGS) layers has been examined by electron microscopy. Successful labeling was carried out as early as two weeks after crush in fish maintained at 20 degrees Centigrade at which time numerous labeled synaptic contacts were seen and this represents the earliest demonstration of specifically labeled synapse formation in this system. Interestingly, many HRP-labeled retinal synapses are concentrated into large discrete clusters consisting of labeled unmyelinated axons, labeled vesicle-containing synaptic profiles and unlabeled post-synaptic processes. The density of HRP-labeled unmyelinated axon bundles and clusters containing HRP-labeled synaptic profiles increased dramatically in goldfish three and four weeks after optic nerve crush.

At 30 days after crush no sublaminar deployment of labeled retinal afferents is evident. In contrast, in normals, quantitative analysis of labeled retinal afferents from overlapping electron micrographs representing a column through the SO-SFGS shows that the primary innervation layer consists of six sublaminae of optic fibers and terminals. Preliminary electron microscopic results indicate that the normal laminar pattern of retinal afferents is discernible at 60 days after crush. Subsequently, six months after crush, the unmyelinated axon bundles have disappeared, large fascicles of labeled myelinated optic fibers are restricted mainly to the SO and the normal sublaminar pattern of labeled retinal synapses is restored.

These findings indicate that the laminar pattern of retinotectal connectivity arises from a reorganization of retinal synaptic clusters and that the laminar reorganization of retinotectal afferents in the SO-SFGS is temporally correlated with the transition from gross to refined retinotopography. The bundling and clustering of early regenerating retinal fibers and terminals implicates direct interactions between retinal fibers during the early phase of retinotectal regeneration.

In addition, we show that optic fibers regenerating under continuous tetrodotoxin blockade of nerve impulse activity form numerous synaptic contacts twelve weeks after crush and these HRP-labeled retinal afferents appear to be deployed in a sublaminar fashion. PHS Grants HD-07029 and NS-16319.

288.8 TECTAL INTERACTIONS INFLUENCE THE RECOVERY FROM INJURY OF GOLDFISH RETINAL GANGLION CELLS. D.W. Burmeister*[1], A.A. Dunn-Meynell*[2] and B. Grafstein[1]. [1]Dept. of Physiology, Cornell University Medical College, New York, NY 10021, and [2]Dept. of Ophthalmology, New York Medical College, Valhalla, NY 10595.

Following axonal injury the retinal ganglion cells (RGCs) of the goldfish exhibit an increase in size, uptake of amino acids, and amount and velocity of axonal transport, reaching a peak when the regenerating optic axons contact the appropriate (contralateral) lobe of the optic tectum, then returning to near normal levels by 12W after injury. Removal of the tectum greatly extends the time required for RGC recovery (Burmeister and Grafstein, 1985, Brain Res 327:45).

When the normal target site for the RGC axons is removed, the regenerating fibers form synapses at alternative sites. For example, when half of an optic tectal lobe is ablated there is a synaptic rearrangement leading to compression of the entire visual field onto the remaining tectal remnant (Gaze and Sharma, 1970, Exp Brain Res 10:171; Murray et al. 1982, J Comp Neurol 209:374).

We examined the RGC changes following either removal of the caudal portion of the optic tectum or after a mid-lobe transverse transection. The resident fibers of the remaining rostral tectum were then either left intact or made to relinquish their tectal termination sites by a crush of the optic nerve. --Following a tectal transection without nerve crush, only the RGCs normally projecting to the caudal tectum (those in the nasal retina) increased in size, reaching a maximum at 2W after injury and then declining to near normal size by 6W. --Following a tectal transection and nerve crush, RGCs in temporal as well as nasal retina initially increased in size and by 12W had declined from peak levels to values 20% larger than normal. --Following caudal tectal ablation without nerve crush, nasal cells alone increased in size initially, and remained 45-50% larger than normal after 12W. --After a caudal tectal ablation and nerve crush, all the RGCs in the retina increased in size. But at 12W after the injury cells in the nasal retina remained 50% larger than normal while cells in the temporal retina were 20% larger than normal.

These results show that the increased competition for synaptic targets in a reduced tectal field led to a prolongation of the regenerative state only among the "foreign" nasal RGCs. Temporal cells recovered from injury equally well in the presence or absence of the caudal tectum and the invading nasal fibers.

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288.9 SYNAPTIC ORGANIZATION OF ANOMALOUS RETINAL TERMINALS WITHIN SOMATOSENSORY THALAMUS AFTER NEONATAL BRAIN LESIONS IN HAMSTERS. G. Campbell* and D.O. Frost. Section of Neuroanatomy, Yale University School of Medicine, New Haven, CT 06510,

If the superior colliculus and dorsal lateral geniculate nucleus (LGd) are ablated and the ventrobasal nucleus (VB) is partially deafferented in the Syrian hamster on the day of birth, the normally transient neonatal retino-VB projection (Frost, J. Comp. Neurol., 230:576, 1984) becomes permanently stabilized and sprouts (Frost, J. Comp. Neurol., 203:227, 1981). This study was undertaken to determine whether the anomalous, permanent retino-VB axons form synapses with VB neurons. In 8 neonatally operated, adult hamsters the anomalous projections were labeled by intravitreal injections of horseradish peroxidase; VB sections were then reacted with modified Hanker-Yates and tetramethyl benzidine protocols and processed for electron microscopy. VB and LGd material from unoperated adult animals with and without eye injections was also examined. Reaction product was clearly visible in retino-VB axons and their terminals. Labeled terminals were of variable size and shape and contained round synaptic vesicles and pale mitochondria. In these respects they were similar to retinal terminals in normal LGd (So et al., Anat. Embryol., 171:223, 1985). Labelled retino-VB terminals were almost exclusively located in well-differentiated glomeruli where they formed Gray type 1 synapses with invaginating spines arising from the proximal dendrites of presumptive thalamocortical relay cells. They also established filamentous and adherens contacts with relay cell dendritic shafts. Some labeled terminals appeared larger and were presynaptic to more invaginating dendritic spines than retinal terminals in normal LGd. In these respects they closely resembled presumptive somatosensory lemniscal terminals in normal VB, although they lacked the dark mitochondria of lemniscal terminals. Boutons containing flattened synaptic vesicles and resembling the likely inhibitory F-boutons of normal rodent thalamus made Gray type 2 synapses adjacent to, or opposite, labeled terminals on the same dendritic shaft. This arrangement is rarely found in normal LGd glomeruli which have presynaptic dendritic appendages as their probable inhibitory component. However, F-boutons are characteristic of normal VB glomeruli which lack presynaptic dendrites. These results demonstrate that in neonatally operated hamsters, anomalous retino-VB axons form well-differentiated synapses with thalamocortical relay cells. Although some anomalous retino-VB terminals resemble retinal terminals within normal LGd, others have synaptic relationships more similar to those of somatosensory lemniscal terminals. This suggests that the differentiation of some retinal terminals is influenced by their targets. Supported by NIH Grant EY03465, March of Dimes Grant 5-417, and a Burroughs-Wellcome Bridging Grant.

288.10 THE ROLE OF THE TARGET IN THE FORMATION OF THE OLFACTORY GLOMERULUS. J.A. Heckroth*, E.E. Morrison, P.P.C. Graziadei and G.A. Monti Graziadei. Department of Biological Science, Florida State University. Tallahassee, FL 32306

The glomerulus in the intact olfactory bulb of mammals is composed of three fundamental elements: a) the input fibres, i.e. the olfactory sensory axons, b) the target, i.e. the dendritic branches of the mitral cells, c) the interconnecting element, i.e. the periglomerular cells. At the LM level the glomerulus can be recognized as a globose structure, some 100 microns in diameter with boundaries well defined from the surrounding brain parenchyma. Previous observations in our laboratory have shown that the glomeruli do not necessarily need the mitral cells or periglomerular cells profiles for their formation. In partial bulbectomies we have observed glomerular structures in the granular layer of the bulb. In total bulbectomy experiments glomeruli have been observed in several regions of the telencephalic cortex. However, while axons originated from the entire sensory sheet of the nasal cavity invariably form glomeruli in several regions of the brain, fragments of the olfactory epithelium transplanted in those regions fail to do so. We have postulated that glomeruli are formed by mutual recognition between complementary sets of sensory axons, originating from different areas of the sensory sheet, even independently of any specific target.

To investigate this hypothesis we have transplanted discrete areas of rat olfactory epithelium or the entire olfactory anlage in four selected regions of rat hosts: 1) the anterior chamber of the eye, 2) the lateral cerebral ventricle, 3) the parietal cortex, 4) the olfactory bulb. Fibre bundles from fragments of the neuroepithelium never formed glomeruli, while fibre bundles comparable in size from the entire nasal anlage were consistently able to form glomeruli. These glomerular formations were different from common neuromataor from axon curls at both LM and TEM levels. At the ultrastructural level they were composed of sensory axon terminals arranged in tangles and showing spurious synaptic contacts between sensory fibres or between the latter and glial profiles. Thus, we can redefine glomeruli as a tangle of sensory axon terminals provided with vesicles and arranged in a discrete, globose formation. It appears that recognition between sensory fibres, even in the absence of any other target, is a prerequisite and sufficient condition for the formation of glomerular structures, so far as complementary populations of sensory axons can intermingle. The mechanisms by which sensory axons specifically recognize mitral cells in normal development seems to be secondary to the primary reciprocal recognition between subsets of primary sensory axons.

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288.11 GAP JUNCTIONS IN THE BRAINSTEM RETICULAR FORMATION: A GOLGI-EM STUDY. J. Quattrochi* (SPON: M. Marin-Padilla). Departments of Neuroscience, Children's Hospital and Neuropathology, Harvard Medical School, Boston, MA 02115.

Five isodendritic magnocellular neurons in the nucleus gigantocellularis of the mouse medullary reticular formation were examined with combined rapid Golgi-EM after fixation with buffered 5% glutaraldehyde and 4% paraformaldehyde. The multipolar somata of these five cells measured approximately 22x34 um with 3-5 dendrites extending from the cell body. Selected cells were cut initially into 2 um serial sections transverse to the longitudinal axis of the brainstem, re-embedded, and selected sections from the series were serially sectioned at 60 nm. Ultrastructural profiles of these de-impregnated cells were identified by the gold particles in their cytoplasm. Each individually gold-toned neuron was examined with particular attention to dendritic synaptic profiles. In addition to synaptic boutons forming symmetric and asymmetric synapses, 9 gap junctions were seen between gold-toned and unimpregnated dendrites. Gap junctions were located 80-200 um from the cell soma. They were found in 4 animals between the ages of 5 and 20 postnatal days. Each demonstrated characteristic ultrastructure and measured 0.2-0.5 um in diameter. All gap junctions were seen along primary dendrites: 6 located distal and 3 proximal to an initial dendritic branch point. One gap junction was seen between two de-impregnated dendrites in linear parallel apposition to each other. Computer reconstruction of 26 serial 60 nm sections of this particular junction site revealed a dendritic contact measuring 1.6 um in length with a single gap junction of approximately 0.3 um in diameter. Ultrastructural evidence of gap junctions hitherto unrecognized on neurons within the brainstem reticular formation is presented. Supported by NIH grants NS20822, NS07264, NS20820.

288.12 USE OF CELL SURFACE-DIRECTED MONOCLONAL ANTIBODIES TO ISOLATE AND STUDY CULTURED NEURONAL SUBPOPULATIONS FROM THE DEVELOPING MOUSE CNS. U. di Porzio, G. Rougon*, J. Mazzetta*, W. Kell* and J.L. Barker. Laboratory of Neurophysiology, NINCDS and Laboratory of Cell Biology, NIMH, NIH, Bethesda, Md. 20205; Institute of Molecular Embryology, CNR, Naples, Italy

Primary cultures of central and peripheral neurons have been extensively used in recent years to study development of functional connections during neurogenesis. For example, we have shown in previous work that target neurons from the striatum and/or cerebellum specifically stimulate uptake and synthesis of catecholamines in the presynaptic dopaminergic or noradrenergic neurons (di Porzio et al., Nature, 288:370, 1980; Dev. Brain Res., 16:147, 1984). A major limitation of primary culture systems is that many cell types survive in vitro with consequent high heterogeneity in the cultures; in many instances the class of neurons under study accounts for a relatively minor proportion of the total cell population. Therefore the isolation and subsequent enrichment in culture of neurons with specific neurotransmitter phenotypes is an important goal in developmental study of the nervous system.

We have used several monoclonal antibodies (MAb) raised in rats immunized against weaver mouse cerebellar membranes that recognize surface glycoproteins differentially expressed in neuronal subpopulations (Rougon et al.,Neurosci., 10:511, 1983; J. Neuroimmunol., 6:411, 1984). The MAbs NSP4 and NSP5 have been used first to identify subpopulations of neurons in cell cultures from the embryonic mouse mesencephalon and brain stem. In these cultures about 15% of the neuronal population appeared to be NSP4+ at early (2 days) and late (10 d) stages in vitro whereas NSP5 recognizes a subpopulation of neurons only in 10 d cultures thus indicating a developmental regulation of the surface antigens recognized by the two MAbs. Flow cytometric analysis with a FACS 440 showed that about 20% of cells freshly dissociated from mouse E13 mesencephalon and E15 basal ganglia were NSP4+. The NSP4+ population sorted by FACS and plated over different substrata showed optimum attachment to a feeder layer of brain astrocytes. Under these conditions the majority of the sorted cells exhibited neuronal morphologies after 2-3 days in culture and exenteded neurites as shown by immunohistochemistry with MAb anti neurofilament. We conclude that NSP4 can be used to separate subpopulations of neurons at various stages of embryonic development. Further studies will establish whether MAbs NSP4 and NSP5 can be used to isolate homogeneous subsets of neurons according to their neurotransmitter phenotype and/or stages of neuronal differentiation.

## ACETYLCHOLINE II

289.1 EFFECT OF PIPERIDINE ANALOGS OF HEMICHOLINIUM-3 ON HIGH AFFINITY UPTAKE OF CHOLINE. J.P. Long*, T.K. Chatterjee*, J.G. Cannon*, and R.K. Bhatnagar, (SPON: W.J. Steele). Department of Pharmacology, University of Iowa, College of Medicine, Iowa City, Iowa 52242

We have reported that quaternary and tertiary analogs of 4-methyl piperidine derivatives of hemicholinium-3 (HC-3) are potent inhibitors of rabbit neuromuscular transmission (Fed. Proceed. 43: 342, 1984). In this study we evaluated the effect of 4-methyl as well as 4-hydroxy derivatives of HC-3 on $Na^+$ dependent high affinity choline uptake (HACU) in rat caudate synaptosomal preparations. At low concentrations (2-4 nM) HC-3 facilitated HACU by 10-15% while at higher concentrations it inhibited HACU with an $IC_{50}$ value of 20 nM. The tertiary analog of HC-3, 4-methyl piperidine derivative ($A_4$), produced a similar biphasic response, an initial uptake facilitation (20-40%) at concentrations between 2 and 8 nM and inhibition at higher concentrations with an $IC_{50}$ value of 40 nM. The quaternary analog of HC-3, N-methyl 4-methyl piperidine derivative ($A_5$), however, did not elicit a biphasic response but potently inhibited HACU with an $IC_{50}$ value of 2.4 nM, a concentration at which both HC-3 and $A_4$ stimulated this uptake. The kinetic characteristics of HACU inhibition by HC-3 and $A_5$ were different also. While HC-3 inhibited HACU competitively, $A_5$ inhibition was noncompetitive with the following apparent $K_m$ and $V_{max}$ values:

| | $K_m$(μM) | $V_{max}$ (pmole/4 min/mg protein) |
|---|---|---|
| Control (n=3) | 1.4 ± 0.06 | 107 ± 12 |
| HC-3-10nM (n=3) | 2.5 ± 0.20* | 112 ± 8 |
| $A_5$-1 nM (n=3) | 1.4 ± 0.09 | 87 ± 7* |

(* Significantly different from control, $P < 0.05$)

The effects of both $A_5$ and HC-3 on HACU inhibition were reversible since washing of the drug-treated synaptosomes with drug-free buffer restored normal HACU. Quaternary and tertiary analogs of 4-hydroxy piperidine derivatives of HC-3, in contrast to 4-methyl derivatives discussed above, were inactive either in affecting rabbit neuromuscular transmission or HACU.

These results indicate that, unlike 4-hydroxy piperidine derivatives, both tertiary ($A_4$) and quaternary ($A_5$) 4-methyl piperidine derivatives of HC-3 are potent inhibitors of $Na^+$ dependent high affinity uptake of choline. Furthermore, $A_5$ may inhibit this choline uptake by a mechanism different from that of HC-3 and $A_4$, and may be useful in further characterizing the mechanism for high affinity uptake of choline in the biosynthesis of acetylcholine. This work supported in part by the U.S. Army Medical Research and Development Command under Contract DAMD-17-83-C-3010.

289.2 CHARACTERISTICS OF [$^3$H]HEMICHOLINIUM-3 BINDING IN RAT BRAIN CAUDATE MEMBRANE PREPARATIONS: EQUILIBRIUM, KINETIC AND COMPETITIVE BINDING STUDIES. T.K. Chatterjee*, J.G. Cannon*, J.P. Long*, and R.K. Bhatnagar. Department of Pharmacology, University of Iowa, College of Medicine, Iowa City, Iowa 52242

Cholinergic nerve terminals have the unique ability to accumulate choline inside nerve terminals via a sodium dependent high affinity uptake system. Hemicholinium-3 (HC-3), a competitive inhibitor of this uptake process, appears to be a sensitive probe to label and characterize this carrier site in vitro. In this study we examined the equilibrium, kinetic and competitive binding characteristics of [$^3$H]HC-3 in twice-washed homogenate of rat brain caudate tissue at 25°C in 50mM $Na^+/K^+$ phosphate buffer, pH 7.4, containing 150mM NaCl. Nonspecific binding was estimated in the presence of 1μM unlabeled HC-3.

Scatchard analysis of equilibrium binding data indicates at least two binding sites for [$^3$H]HC-3, one with high affinity ($K_d$ 1.5nM) and low capacity ($B_{max}$ 80 fmol/mg protein) and other with low affinity ($K_d$ 30nM) and high capacity ($B_{max}$ 400 fmol/mg protein). The dissociation of [$^3$H]HC-3 from its binding sites, induced by infinite dilution with buffer, proceeded in a multi-exponential manner with a fast and a slow dissociating component and the dissociation rate was accelerated when unlabeled HC-3 was included in the diluting buffer. The association of varying concentrations of [$^3$H]HC-3 with its binding sites was also non-linear. The displacement studies demonstrated that HC-3 and choline displace [$^3$H]HC-3 with a Hill coefficient (nH) of less than unity. At 12nM concentration of [$^3$H]HC-3 the $IC_{50}$ values for HC-3 and choline for displacement were 30 nM and 0.6 μM respectively, while at 1 nM [$^3$H]HC-3, these values were 8 nM and 0.15 μM, respectively.

These results suggest the presence of at least two binding sites for HC-3. The accelerated dissociation of [$^3$H]HC-3 by competitive displacement and nH of < 1 for [$^3$H]HC-3 displacement by HC-3 and choline suggest the possibility of a ligand induced occupancy dependent conversion of the high affinity binding state to low affinity one. Since the affinities of HC-3 and choline for the low affinity site correspond to their affinities in affecting choline uptake process, the low affinity state, induced by ligand occupancy, may represent the active choline carrier site. This work supported in part by the U.S. Army Medical Research and Development Command under Contract DAMD-17-83-C-3010.

289.3 RAPID REGULATION OF $^3$H-HEMICHOLINIUM-3 ($^3$H-HCh3) BINDING SITES IN THE RAT CEREBRAL CORTEX AND STRIATUM. P.R. Lowenstein, B. O'Hara* and J.T. Coyle. Dept. of Neuroscience. Johns Hopkins University School of Medicine, Balto., MD 21205.

The velocity of the sodium-dependent high affinity choline uptake (SDHACU) is regulated by the antecedent activity of cholinergic neurons, consistent with SDHACU being the rate-limiting step in the synthesis of acetylcholine (ACh). The mechanisms responsible for the rapid changes in SDHACU velocity have not yet been elucidated. Recent studies indicate that [$^3$H]HCh-3, a potent inhibitor of SDHACU, labels the choline carrier in brain homogenates. In order to characterize the mechanisms involved in SDHACU regulation, we studied the acute effects of drugs known to affect cholinergic neuronal activity on [$^3$H]HCh-3 binding in the cerebral cortex and corpus striatum of the rat. The drugs were administered by IP injection and the rats were sacrificed 30 min later. Extensively washed membranes were prepared from the cortex and the striatum for specific binding of [$^3$H]HCh-3 (10uM). Results are mean + SEM of 4 or more rats. Atropine (20mg/kg) and scopolamine (5mg/kg), two cholinergic antagonists which increase ACh turnover and SDHACU, elicited an 85+11% and 63+4% increase respectively in [$^3$H]HCh-3 binding in the cortex. In the striatum, atropine increased the binding by 66+5%, while oxotremorine (2mg/kg), a cholinergic agonist which decreases cholinergic nerve activity and SDHACU, decreased the binding by 43+18% in the cortex and 32+9% in the striatum. Cholinergic neurons in the striatum, but not in the cortex, are under inhibitory dopaminergic control. Treatment of rats with haloperidol (4mg/kg, 1hr), a dopaminergic antagonist which increases striatal ACh turnover and SDHACU, increased total binding in the striatum by 23+5%. Pentobarbital (65mg/kg), a general anesthetic agent, decreased total binding by 38+18% in the cortex and 30+8% in the striatum, while pentylenetetrazole (75mg/kg), a convulsant agent, increased total binding in both the cortex (17+4%) and striatum (26+3%) by the time of convulsion's onset. Scatchard analysis of saturation isotherms for [$^3$H]HCh-3 under different treatment conditions revealed that alterations in binding resulted from changes in the total number of binding sites ($B_{max}$) and not the $K_D$, which remained constant ($K_D$=4uM). These results show that different types of drugs, known to affect cholinergic function in specific (e.g., atropine) or nonspecific (e.g., pentobarbital) ways are able to affect the density of high affinity [$^3$H]HCh-3 binding sites in accordance with their overall effects on cholinergic nerve activity. Thus, not only SDHACU but also [$^3$H]HCh-3 binding sites might represent a reliable index of cholinergic neuronal activity in vivo. In addition, a rapid up or down regulation of the total number of choline carrier sites on the cholinergic nerve terminals appears to account for the changes in SDHACU activity.

289.4 AGE RELATED RE-APPEARANCE OF PARTIAL BASAL FOREBRAIN CHOLINERGIC DEFICITS. M. Brown*, C.F. Hohmann, P.R. Lowenstein, W. Meck, G.L. Wenk and J.T. Coyle. Johns Hopkins Univ., Balto., MD 21205 and Dept. of Psychol., Brown Univ., Prov., R.I. 02912 (SPON: L.Tune).

Lesions of basal forebrain cholinergic nuclei have recently received attention as a potential animal model for selected pathological aspects of Alzheimer's Disease (AD). Loss of cholinergic cells in the ventral globus pallidus correlates with a significant decrease in cortical cholinergic markers, such as choline acetyltransferase (ChAT) and levels of high affinity choline uptake (HACU). The specific activity of ChAT is assumed to correlate with the density of cholinergic innervation to a brain region. HACU and the specific binding of [$^3$H]Hemicholinium-3 (HCh-3) to the choline carrier are under dynamic controls that reflect the recent history of cholinergic neuronal firing. In rats that receive unilateral lesions in the basal forebrain cholinergic system, levels of ChAT activity and HACU gradually recover.

The present study shows that the post-lesion recovery of cortical ChAT activity is temporary. ChAT activity and the specific binding of [$^3$H]HCh-3 were measured in neocortex 4 months and 14 months after unilateral basal forebrain injections of the perikaryal specific neurotoxin, ibotenic acid. By 4 months after the ibotenate lesion, the activity of ChAT on the lesioned side had recovered to that of the contralateral unlesioned cortex (89 + 4 S.E.M. vs 86 + 2 S.E.M. nmoles/mg/hr, N=4). However, animals lesioned 14 months prior to enzyme measurements showed a 30% decrease of ChAT activity in the cortical hemisphere ipsilateral to the ibotenic acid injection ($p < 0.001$, N=4). The specific binding of [$^3$H]HCh-3 did not differ significantly between cortical hemispheres at either time post-lesion. Since the re-appearance of ChAT losses seemed to coincide with aging in the lesioned animal, we examined how aging in this rat strain may affect cholinergic marker levels. ChAT activity, QNB binding, HACU and [$^3$H]HCh-3 binding were monitored in cortex of animals aged 3 to 30 months. The specific activity of ChAT did not change with age. Both HACU and specific binding of [$^3$H]HCh-3 increased with age and QNB binding showed a slight concomitant decrease. It thus appears that cholinergic activity in cortex increases with aging while the number of cholinergic elements in cortex remains constant. We hypothesize that the re-appearance of post-lesion ChAT losses in aging animals is a consequence of cholinergic neuron overactivity. Initial recovery of ChAT levels in unilaterally lesioned animals appears to be a consequence of sprouting. But, as the lesioned rats age, fewer neurons must maintain increased firing levels in a more extended axonal territory. Some neuronal processes may not be able to sustain these levels of activity and degenerate. The present finding may have important implications for the time of disease onset in AD or Down's syndrome.

289.5 QUANTITATIVE HISTOCHEMISTRY OF BRAIN ACETYLCHOLINESTERASE IN HUMANS AND AGED RATS. A. Biegon, M. Wolff*, V. Greenberger* and M. Segal*. Isotope Department, The Weizmann Institute of Science, 76100 Rehovot, Israel.

Acetylcholinesterase (AChE) is an important marker of the cholinergic system. Selective decreases in AChE activity are observed in the brains of patients who died of Alzheimer's disease. In the rat, it was not clear whether aging is accompanied by changes in AChE activity, mainly, we believe, because highly localized changes in small nuclei (such as the medial septal nucleus) were technically hard to determine using available methodologies. To facilitate quantitative and reproducible measurements of AChE in discrete brain regions, we have adapted a AChE histochemical procedure for quantitative use. Mashed rat cerebelli were mixed with known quantities of a purified AChE preparation, frozen and thin sectioned on a cryostat at -20°C. Experimental brains were frozen and cut under the same conditions. The brain sections and standards underwent histochemical staining simultaneously and a computerized image analysis system was used to construct a standard curve and analyze the densities of staining in experimental tissue. The distribution of AChE in coronal sections of human brains collected post mortem was quantified. In a group of six normal middle aged subjects, inter individual variation was small. In a single case of suicide by parathion ingestion (an AChE inhibitor) a large but anatomically heterogeneous decrease in AChE staining was observed. Using this technique, we have measured AChE in the rat brain nuclei containing cholinergic neurons (medial septal nucleus and ventral pallidum) and several terminal areas. Eight aged (31 months old) and eight young (4 months old) rats were compared. We observed a substantial (10% to 70%, mean 33%, $p<0.003$) decrease in AChE activity in the ventral pallidum. Significant decreases were also found in the medial septal nucleus, anterior cingulate cortex and the dentate gyrus of the hippocampus. No changes were observed in the CA fields of the hippocampus, caudate nucleus and olfactory tubercule. The AChE levels in individual rats showed a significant positive correlation with performance in a water maze, indicating the heterogeneity of spatial memory deficits in aged rats and the involvement of the cholinergic system in cognitive processes. Quantitative histochemistry is currently being applied to a detailed analysis of AChE content in brains of Alzheimer's disease patients post mortem.

289.6 CHOLINERGIC CORTICAL INNERVATION: A CORRELATIVE STUDY OF CHOLINE ACETYLTRANSFERASE-IMMUNOREACTIVE VERSUS ACETYLCHOLINESTERASE-POSITIVE FIBER DISTRIBUTION. A. Lysakowski[1], B.H. Wainer[1], D.B. Rye[1], G. Bruce[2], and L.B. Hersh[2]. [1]Dept. Pharmacological and Physiological Sciences, The University of Chicago, Chicago, IL 60637; [2]Dept. Biochemistry, University of Texas Health Sciences Center, Dallas, TX 75235.

Cholinergic innervation of cerebral cortex contributes to cognitive function, yet little is known concerning the precise anatomical organization of this system. Previous studies have utilized acetylcholinesterase (AChE) histochemistry to visualize putative cholinergic cortical fibers. In the present study, a recently developed polyclonal antiserum against the specific cholinergic marker, choline acetyltransferase (ChAT), was employed to localize ChAT-immunoreactive (ChAT-IR) fibers in different areas of rat cerebral cortex. In adjacent sections, acetylcholinesterase-positive (AChE+) fibers were visualized histochemically (Hedreen et al., J. Histochem. Cytochem., 33:134-140, 1985).

In 16 cytoarchitectonic areas examined, several unique laminar patterns of ChAT-IR fibers were observed. In some of these areas, distinct differences were noted when compared with the distribution of AChE+ fibers. Most neocortical areas exhibit tangentially-oriented bands of both fiber types in layer I. Primary sensory cortices also exhibit bands of ChAT-IR and AChE+ fibers in layer IV. However, in the barrel field, ChAT-IR fibers form "septa," leaving ChAT-poor "hollows" within the barrels, while AChE+ fibers ramify within the "hollows," leaving the "septa" relatively clear. Motor cortex exhibits a homogeneous pattern of ChAT fibers in layers II-VI. In medial areas, cingulate cortex contains thick ChAT-IR and AChE+ tangential fibers in layers I and VI, while marked differences are noted in retrosplenial cortex. ChAT-IR fibers are dense in layers I and IV-VI and scarce in layers II-III. The latter two layers are primarily populated by small, densely-packed cells and contain significant numbers of ChAT-IR cell bodies. In contrast, AChE+ fibers are very dense in layers II-III and much less dense in other layers. In hippocampus, ChAT-IR fibers are prominent as narrow bands immediately above and below the granule and pyramidal cell layers. AChE+ fibers are also dense adjacent to the cell layers, but show additional banding within the fiber layers.

In summary, regional and laminar variations in cortical cholinergic fibers may represent different functional circuitries. In addition, AChE varies from ChAT in its fiber distribution and may not be a reliable marker for cortical cholinergic innervation.

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289.7 IDENTIFICATION OF CHOLINERGIC NEURONS IN RAT RETINA. C.T. Lin, Y.-F. Xu*, J. W. Liu*, H.S. Lin*, S. Wei*, J.-Y. Wu. Department of Physiology, College of Medicine, The Penn State University, Hershey, PA 17033

Choline acetyltransferase (ChAT) was purified from rat brain to homogeneity. The purified ChAT preparations were used for the production of polyclonal antibodies as well as monoclonal antibody. Both polyclonal and monoclonal antibodies were characterized by enzyme inhibition test and by Western blot transfer method and used to localize ChAT in the rat retina by indirect immunoelectron microscopy. Rats were perfused with 4% paraformaldehyde and 0.1% glutaraldehyde mixture. Each eye ball was dissected and retina isolated. The retina was further fixed in the same fixative for another 2 h and immersed in 4% paraformaldehyde overnight. Fifty micrometer sections were obtained from a vibratome. Sections were incubated with antibodies against ChAT, followed by peroxidase labeled second antibody, and finally with peroxidase substrate. At light microscopic level, reaction product (RP) was seen mainly in the inner plexiform layer (IPL), some in the inner nuclear layer (INL) and the outer plexiform layer (OPL), and occasionally in the outer nuclear layer (ONL). In the INL, some horizontal cells, amacrine cells and possible bipolar cells were stained. At electron microscopic level, in the IPL, electron dense RP was seen in some amacrine terminals and bipolar terminals. The stained amacrine terminals were seen to make synapse with other stained and unstained amacrine and bipolar terminals. Similarly, the stained bipolar terminals also made synaptic contact with some stained and unstained amacrine and bioplar terminals. In the OPL, some rod spherules and cone pedicles also contained RP. In both IPL and OPL, RP was seen to be associated with presynaptic vesicles and presynaptic membrane. RP in the stained cell bodies in ONL and INL was seen to be associated with free polysomes and other cellular organelles. These findings suggest that some rod and cone cells in the ONL, some horizontal cells, bipolar cells and amacrine cells in the INL may use acetylcholine as neurotransmitter. (Supported by EY-05397, NS-20978 and NS-20922).

289.8 **FREQUENCY DEPENDENT POTENTIATION OF A PRESUMPTIVELY CHOLINERGIC FOREBRAIN INPUT TO THE RAT OLFACTORY BULB.** W.T. Nickell and M.T. Shipley. Department of Anatomy and Cell Biology, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267

Anatomical studies have demonstrated an extensive, largely cholinergic projection from the nucleus of the horizontal limb of the diagonal band (DB) to the olfactory bulb (OB) in the rat. Very little is known, however, about the functional characteristics of this or any other basal telencephalic cholinergic projection system. The DB→OB projection terminates in a highly specific laminar pattern which greatly facilitates the analysis of pre-and post-synaptic events. We have stimulated the region of the diagonal band using bipolar electrodes while recording from OB of anaesthetized rats (chloral hydrate) with micropipettes. In some cases mitral cells were stimulated antidromically by electrodes placed in the lateral olfactory tract (LOT) to test for interaction with DB potentials.

Single DB shocks produce a biphasic (positive-negative) potential in the internal plexiform layer (IPL). This potential develops immediately after the recording electrode passes the mitral cell layer (MCL). A potential of opposite sign is present in the external plexiform layer. At low stimulation frequency these potentials show no detectable facilitation or depression. Single shocks have no influence on potentials resulting from LOT stimulation.

However, when the DB is stimulated for >10 seconds at 10 Hz or greater, dramatic changes are produced in OB. After a few seconds of DB stimulation, there is a period of inhibition of the potential lasting 1-3 seconds. Then, abruptly, the amplitude of the DB potential increases to an extraordinary degree. Although both phases of the potential are increased, the negative phase increases so much in magnitude and time-course as to suggest the initiation of a new process. If stimulation continues for several seconds after onset of the potentiation, the bulb is sometimes left in an oscillatory state in which potentials resembling the negative phase of the DB potential occur spontaneously. This potentiated state persists for 5-10 seconds and can be maintained for long periods by stimulation at low frequency (1-2HZ). Stimulation of LOT during the potentiated state causes the appearance of a long-latency negative going potential not present in the unpotentiated record. This potential has similar latency and time course to the negative phase of the DB potential during potentiation.

The features of this potentiation are consistent with those of muscarinic synapses. If the cholinergic nature of the effect is confirmed, the DB→OB pathway will be an important model for cholinergic influence on cortical structures.

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289.9 EFFECT OF INTRA-CORTICAL INJECTION OF DFP ON CHOLINERGIC NEUROCHEMISTRY AND PASSIVE AVOIDANCE BEHAVIOR IN THE RAT. S.E. Robinson, H. Muller-Kahle* and M.A. Rice*. Department of Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298-0001.

Peripheral administration of the irreversible cholinesterase inhibitor DFP has been reported to enhance retention of passive avoidance behavior in conjunction with reduced muscarinic receptor binding in the cerebral cortex (Lerer et al., Pharmacol. Biochem. Behav. 21:467, 1984). In order to determine if DFP can enhance memory by action in the cerebral cortex, we have investigated the effect of intra-cortical injection of DFP into the parietal cortex on passive avoidance behavior. Male Sprague-Dawley rats (150-250 g) were implanted bilaterally with guide cannulas in the parietal cortex (AP interaural + 9.7 to 8.7 and L ± 5.5). One week later, 122 nmol of DFP in a total volume of 0.75 µl or 0.75 µl of artificial CSF:emulphor (9:1) vehicle was injected in the following way. An internal cannula was lowered through the guide cannula to a depth of 6.5 mm from the skull surface, half the volume infused over a period of 1 min and then the internal cannula was raised 3 mm and the remainder of the dose infused over 1 min. Thus, maximal inhibition of cholinesterase activity in the cortex with minimal inhibition of enzyme activity outside of the cortex was achieved. Cholinesterase activity, as measured by the method of Ellman et al. (Biochem. Pharmacol. 7:88, 1961), in the cortex was 42%, 43% and 51% of control at 20 min, 1 hr and 24 hr following DFP administration, whereas cholinesterase activity was not inhibited by more than 38% in any other brain area examined (striatum, medulla/pons, hypothalamus, hippocampus, amygdala). A separate group of animals were tested in a passive avoidance paradigm in a two-compartment shuttle-box. Animals were allowed a 30 sec period in which to explore the illuminated compartment of the shuttle box, after which the animals were allowed access to the dark compartment. Animals received a brief 1 mA inescapable shock when they crossed to the dark side. A maximum crossover latency of 300 sec was allowed. A total of 10 trials was performed and rats were allowed 180 sec in their home cage between trials. Twenty min after intracerebral injection there was no significant difference between the DFP and vehicle-treated rats in acquiring the passive avoidance behavior or in the initial crossover latency. Atropine (total dose 30 nmol, injected as described for DFP above) also does not appear to affect acquisition of passive avoidance behavior or initial crossover latency 20 min after injection into the parietal cortex. Behavioral studies are also being performed 1 hr and 24 hr following DFP administration. (Supported by contract number DAMD 17-83-C-3183).

289.10 AGING AND ACETYLCHOLINE-RELEASE FROM RAT CORTICAL SYNAPTOSOMES AND ATRIAL MINCES. E.M. Meyer, S.P. Baker, F.T. Crews, and K. Larsen* Dept. of Pharmacology and Therapeutics, U. Florida, Gainesville.

Aging consistently reduces the depolarization-induced, calcium-dependent release of newly synthesized ACh but not that of other transmitters from rat brains. In order to ascertain whether this reduction in transmitter release reflects changes in voltage-dependent calcium influx or to a step distal to calcium uptake, we studied the effects of several secretagogues on calcium-dependent ACh release from rat cortical synaptosomes derived from 6 or 24 month old male Sprague-Dawley albino rats. $K^+$-depolarization increased the release of newly synthesized ACh from synaptosomes in both groups of animal but released significantly more transmitter in the younger tissues. A23187, the calcium ionophore that bypasses voltage-dependent calcium uptake, increased ACh-release to a greater extent in the younger group at submaximal concentrations (up to 10 ug/ml), but the maximal release induced by 20 ug/ml of the drug was identical in both groups of animals. No age-related difference in the $^{45}Ca^{2+}$ uptake induced by any concentration of A232187 was observed. Ouabain, which acts by mobilizing intracellular calcium ions in the absence of extracellular calcium, increased ACh release to a similar extent in both age-groups. 4-Aminopyridine, which blocks potassium channels and protracts depolarization-induced release of ACh, increased ACh release to a greater extent in the older animals than in the younger ones. None of these age-related differences in the release of ACh were due to changes in the synthesis or levels of the transmitter. Further, no age-related difference in the muscarinic inhibition of ACh release was observed. Taken together, these results suggest that during aging, brain ACh release is attentuated by a reduction in the potency but not efficacy of calcium ions at intracellular release-triggering sites, that larger stores of intracellular calcium ions may be available for mobilization in the older tissues, and that 4-aminopyridine may be useful for selectively increasing ACh release in senescent brains.

Atrial presynaptic cholinergic activity was affected differently by aging than the brain activity was. The 24-month old atria had significantly lower rates of high affinity choline transport activity, synthesized less ACh, and released less of the transmitter in response to 50 mM $K^+$ depolarization than the 6 month old tissues did. The ability of oxotremorine (100 uM) to inhibit ACh release via presynaptic receptors in this tissue was also significantly attenuated by aging in this tissue. These results are consistent with an age-induced reduction in presynaptic cholinergic function in the parasympathetic innervation of the heart, as opposed to the more selective reduction in the potency of calcium ions at intracellular release-triggering sites in brain cholinergic neurons.

290.1 BIOPHYSICAL ALTERATIONS OF RABBIT HIPPOCAMPAL NEURONS STUDIED IN VITRO AFTER CONDITIONING. J.F. Disterhoft, D.A. Coulter* and D.L. Alkon, Laboratory of Biophysics, NINCDS, Marine Biological Laboratory, Woods Hole, MA 02543.

Young adult male albino rabbits were trained in the nictitating membrane/eyeball retraction conditioning paradigm using white noise CS and periorbital shock US in a short-delay paradigm. The conditioned group was well trained (three 80 trial sessions). The pseudoconditioned group received three sessions of explicitly unpaired, randomized CSs and USs.

Intracellular recordings were made from hippocampal CA1 pyramidal neurons within brain slices prepared one day following the last training session. Surface slices were prepared and maintained using standard techniques. All neurons included (19 conditioned, 19 pseudoconditioned, and 22 naive) had stable penetration, at least 60 mV impulse amplitudes and 20MΩ input resistances. A marked reduction in the afterhyperpolarization (AHP) following an impulse was apparent for conditioned (X=1.0 mV) as compared to the pseudoconditioned (X=1.8 mV; p<.001) and naive (X=1.9 mV; p<.001) neurons. The AHP has previously been attributed to activation of a $Ca^{++}$dependent outward $K^+$ current, $I_C$. This conditioning-specific difference could not be accounted for by differences of input resistance and resting membrane potential also observed. The distribution of AHP amplitudes for the conditioned group included a new lower range of values for which there was no overlap with the other groups. The percentage of conditioned cells in this new group, ~55%, was comparable to that previously observed in vivo to show increased excitability (in response to conditioned stimulus presentations) as measured with extracellular recordings (Berger, T.W. et al., J. Neurophysiol., 50: 1197, 1983. The conditioning-specific reduction of AHP may be due to reduction of $I_C$ as previously shown for neurons from conditioned Hermissenda (Alkon, Science, 226: 1037, 1984).

The amount of sag in the voltage response after injection of large hyperpolarizing pulses was also reduced. The magnitude of sag was significantly less for the conditioned (X=1.7 mV) as compared to the pseudoconditioned (X=3.1 mV; p<.01) and the naive (X=5.4 mV ; p<.001 neurons). The pseudoconditioned group was reduced from the naive (p<.001) so the sag effect was not conditioning-specific.

Our experiments demonstrate that ionic alterations, intrinsic to the hippocampus and not dependent upon other brain regions for their expression, are stored in CA1 pyramidal neurons after conditioning. These alterations are present even in in vitro brain slices separated from the rest of the brain circuitry.

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290.2 CONDITIONING-SPECIFIC REDUCTION OF CA1 AFTERHYPERPOLARIZATION AMPLITUDE AND DURATION IN RABBIT HIPPOCAMPAL SLICES. D.A.Coulter*, M.Kubota*, J.W.Mooret, J.F.Disterhoft§, and D.L.Alkon. Lab. Biophysics, NINCDS-NIH, MBL, Woods Hole, MA 02543; †Dept. Psychol., Univ. Massachusetts, Amherst, MA 01003; and §Dept. Cell Biol. & Anat., Northwestern Univ. Med. Sch., Chicago, IL 60611.

Hippocampal slices were prepared from rabbits one day after training with the nictitating membrane conditioning paradigm for 3 days, 80 trials/day. The CS was a binaural 400 msec tone, coterminating with the UCS, a 100 msec, 1-2 mA periorbital shock train. Pseudoconditioned animals received an equal number of randomized explicitly unpaired stimuli. Intracellular recordings from CA1 pyramidal cells of submerged 500 μm slices (as opposed to fluid interface slices of the companion study, Disterhoft et al., 1985) were included here if impulse amplitudes were $\geq$ 70 mV and if the resting membrane potential was constant during the entire protocol (20-45 min). Among all cells included (N=75) there were no significant differences in membrane potential or input resistance between groups. The afterhyperpolarization (AHP) following a positive current pulse, previously shown to be due in part to activation of a $Ca^{2+}$-dependent $K^+$ current, was greatly reduced by $Co^{2+}$ and $Cd^{2+}$ (N=5, 10 respectively) and by carbachol (N=14). The AHP following a 100 msec positive pulse was significantly lower for conditioned as compared to pseudoconditioned and naive animals when the pulse elicited 4 impulses (cond. $\bar{X}$=-3.43 mV, pseud. $\bar{X}$=-4.9 mV, p<.01; naive $\bar{X}$=-4.52, p<.025, one-tailed t-test), when the pulse elicited 3 impulses (cond. $\bar{X}$=-2.71, pseud. $\bar{X}$=-3.88, p<.01; naive $\bar{X}$=-3.48, p<.025) and, to a lesser degree, when the pulse elicited 2 impulses (cond. $\bar{X}$=-2.16, pseud. $\bar{X}$=-2.90, p<.025; naive $\bar{X}$=-2.51, N.S.). This trend continued but was not significant for pulses eliciting 1 impulse. No significant differences occurred between pseudoconditioned and naive cells for any of the positive pulses used. These findings were consistent with a significantly different distribution for the conditioned cells (vs. pseudoconditioned and naive) in both the AHP amplitude (p<.025, df=4, Chi-square) and duration (p<.02, df=4). Conditioned cells (as compared to pseudoconditioned and naive cells) had many more AHP amplitudes $\geq$-3.0 mV and much fewer $\leq$-6.0 mV and many more AHP durations < 2.0 sec and much fewer $\geq$ 3.5 sec. A clear relationship was found between AHP amplitude (and duration) and the ability of a cell to sustain impulse responses to repetitive activation (by positive 100 msec pulses) of varying frequencies, suggesting the functional importance of these conditioning-specific AHP reductions. In summary, conditioning-specific reduction of a $Ca^{2+}$-dependent $K^+$ current, $I_C$, can contribute to long-term storage of a learned association in the rabbit as was previously shown for $I_C$ (as well as an early current, $I_A$) in the mollusc Hermissenda (cf. Alkon, Science 226, 1037, 1984).

290.3 REVERSIBLE INACTIVATION OF THE HIPPOCAMPUS: EFFECTS FOLLOWING MEMORY REACTIVATION. Douglas C. Smith and James Talman*, Developmental Biopsychology Program and School of Medicine, Southern Illinois University--Carbondale, IL 62901

Lidocaine is a local anesthetic which, when injected directly into the brain, completely and reversibly inactivates all neural activity in the area affected. We have previously presented evidence that bilateral inactivation of the hippocampus (HC) immediately following one-trial inhibitory avoidance training results in deficits, but not complete amnesia, when retention is tested either 60 sec. or 24 hr. later (Smith et al., Soc. Neuroscience Abst., 1983). An even more robust effect is obtained when the HC is inactivated immediately prior to a 24-hr retention test. With this paradigm, rats demonstrate a complete retrieval failure, crossing over into the dark with latencies comparable to those found prior to training (Smith and Talman, Soc. Neuroscience Abst., 1984).

We now report the effects of HC inactivation following memory reactivation. In a one-trial inhibitory avoidance task, 20 Long Evans rats received 1.5 ma, 1.0 sec shock immediately upon entering the dark side from the lighted side of a two-compartment alley. In one group (pre-retrieval), 1 ul of lidocaine was delivered bilaterally to the HC immediately prior to the 24 hr retrieval trial and median latency to enter the dark was 8.3 sec. The other group (reactivation) was placed in the lighted side of the alley 24 hrs after training and demonstrated perfect retention (median latency, 300 sec = ceiling). These animals were then removed from the apparatus, bilaterally injected with lidocaine into the HC and returned for another retention test, and again demonstrated perfect retention (median latency = 300 sec.).

Several conclusions are possible from these results. First, the finding that the subjects in the reactivation group remained in the lighted side of the alley following lidocaine injections into the HC indicates that these injections do not have their effects via some nonspecific motor affect. Thus, the failure of the pre-retrieval group to demonstrate memory for the task must be due to a retrieval failure, and not to any motor activation or failure to be able to inhibit a response. Further, these results suggest that once a memory has been retrieved, the hippocampus is no longer necessary for the continued expression of the memory.

290.4 THE CLASSICALLY CONDITIONED RABBIT NICTITATING MEMBRANE RESPONSE: EXCITATORY AND INHIBITORY CONDITIONED ACTIVITY FROM SINGLE UNITS IN THE BRAIN STEM. J.E. Desmond and J.W. Moore, Dept. of Psychology, Univ. of Massachusetts, Amherst, Mass. 01003

The classically conditioned rabbit nictitating membrane (NM) response is a widely adopted model system for studying the physiology of learning and memory. New Zealand albino rabbits were trained to partially discriminate between a reinforced conditioned stimulus (CS+) and a nonreinforced CS- (tones of 1200 or 600 Hz,75 dB SPL). The unconditioned stimulus was electrostimulation to the periorbital region. Rabbits were anesthetized and prepared for subsequent electrophysiological recording. A stimulating electrode was implanted into the right accessory abducens nucleus (AAN), the principal nucleus mediating the NM reflex. A recording chamber was cemented in place over a small hole on the right side of the skull.

Following recovery, single units in the brain stem were recorded extracellularly in the awake restrained animal during CS+ and CS- presentations. Incomplete discrimination resulted in conditioned response (CR) and non-CR trial types for each CS. Units were tested for antidromic response to stimulation of the AAN electrode.

Statistical tools were developed to investigate CR vs. non-CR unit activity. A binomial method suggested by Dorrscheidt (Brain Res., 220: 397, 1981) was used to compare rates of firing for CR and non-CR trials during identical time periods. Cross correlations of unit activity with the CR, or CR first derivative, were useful in determining lead/lag relationships of unit activity and behavior. CR onset-dependent unit activity was examined via response histograms, which use the CR onset time for each trial as the reference point. Spikes are accumulated in bins around this reference. Multiple regression was also used to assess trial by trial spike/CR relationships. Variables of interest were CR onset latency, CR magnitude, number of spikes during CS period, mean spike time during CS period, and standard deviation of spike time.

Units displaying both CR-related excitation and CR-related inhibition were found. Excitatory units with activity leading the behavior were located in proximity to the motor trigeminal nucleus, including the supratrigeminal region (see Desmond et al., Brain Res. Bull., 10: 747, 1983) or dorsomedial to the brachium conjunctivum. Lead times were sufficiently long for causal involvement, typically ranging from 30-100 ms. Inhibitory units leading the CR were predominately located in dorsal and dorsomedial aspects of nucleus reticularis pontis oralis (RPO). One case of antidromic activation was observed in an excitatory cell in dorsolateral RPO. However this cell probably fired concurrently with the CR.

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290.5 CLASSICAL CONDITIONING OF THE RABBIT NICTITATING MEMBRANE USING STIMULATION OF THE DORSOLATERAL PONS AS A CS. D.G. Lavond, J.E. Steinmetz, D.J. Rosen*, G.J. Beekman & R.F. Thompson. Dept of Psychology, Stanford University, Stanford, CA 94305.

A number of theories have suggested a role for the cerebellum in learning and memory (Albus, Marr, Ito, Eccles). Recent studies have demonstrated an essential role of the cerebellum in classical conditioning. Lesion of the interpositus nucleus prevents or abolishes learning (conditioned responses) without affecting the unconditioned response. Lesion of the inferior olive (climbing fibers) prevents learning and causes extinction in well trained animals, agreeing with the suggestion that the IO provides "reinforcing" input to the cerebellum concerning the unconditioned stimulus. If the cerebellum is the site of neuronal plasticity responsible for learning and memory --the "engram"-- then information concerning the CS and UCS must converge and integrate there into a coherent response (i.e., CR). The present study asks whether stimulation of the mossy fiber afferent from the dorsolateral pontinue nucleus (DLPN) can act as an effective CS when paired with corneal airpuff UCS.

Adult male New Zealand White rabbits were anesthetized and implanted with bipolar stimulating electrodes in the DLPN or mossy fibers. Training consisted of 108 trials per day in which stimulation near the DLPN (CS, 200 Hz, 350 msec, 60 uA) was paired with coterminating corneal airpuff as the UCS (2.1 $N/cm^2$, 100 msec). Group one (N=5) was given paired CS/UCS training to criterion (8 CRs out of 9 consecutive trials) and overtrained. They were then given 4 days of CS-alone extinction followed by 2 days of unpaired CS and UCS trials. Finally, they were retested with paired conditioning. Group two (N=4) was given CS-alone and unpaired experience before paired conditioning. Finally, lesions were made of the cerebellar interpositus nucleus, the rabbits given a few days of recovery, and were then retested for 4 days.

Both groups learned in an average of about 100 trials. Extinction and spontaneous recovery were observed in Group one over days. There was no evidence of nonassociative effects such as sensitization during CS-alone and unpaired training. Lesions of the interpositus nucleus abolished the CRs with no signs of recovery of CRs in 4 days of retraining.

The present study demonstrates that stimulation of DLPN or of mossy fibers can be an effective CS for training an eyeblink response. Furthermore, the CR pathway must involve the cerebellum because lesions there abolish the response. We suggest that the cerebellum is the site of plasticity.

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290.6 CLASSICAL CONDITIONING OF SKELETAL MUSCLE RESPONSES WITH MOSSY FIBER STIMULATION CS AND CLIMBING FIBER STIMULATION US. J. E. Steinmetz, D. G. Lavond, and R. F. Thompson. Dept. of Psychology, Stanford University, Stanford, CA, 94305.

Recent studies have demonstrated that the rabbit eyelid can be classically conditioned when a mossy fiber stimulation CS is paired with an airpuff US (Steinmetz et al., Bull. Psychon. Soc., 1985) and when a tone CS is paired with a climbing fiber stimulation US (Mauk & Thompson, Neurosci. Abst., 1984). The present experiment was an attempt to classically condition skeletal muscle responses with a mossy fiber stimulation CS and a climbing fiber stimulation US.

Stimulating electrodes were implanted into the right dorsolateral pontine nucleus (DLPN) and the right dorsal accessory olive (DAO) of six anesthetized rabbits. The stimulating electrodes were guided into position by recording potentials from cerebellar cortex evoked by single pulse stimulation of the DLPN and DAO. After 1 wk, classical conditioning was begun. Training involved pairing a 350 msec train of DLPN stimulation (60-90 uA, .1 msec, 200 Hz) with a coterminating 100 msec train of DAO stimulation (200-400 uA, .1 msec, 100-400 Hz). The DLPN-CS initially produced no discernable movements while the DAO-US produced a discrete eye blink in 3 animals and a discrete head turn in 3 animals. Rabbits were trained to a criterion with daily sessions of 108 trials, overtrained with an additional session, then given CS-alone or explicitly unpaired extinction training. Paired presentations of the DLPN-CS and DAO-US produced fairly rapid acquisition of the conditioned eyelid or head turn response. Subsequent extinction training abolished the conditioned response with unpaired CS-US presentations producing more rapid extinction than CS-alone presentations. Histological examination of the stimulation sites revealed that US electrodes were positioned in rostromedial portions of the DAO in eye blink animals and in slightly more rostrolateral portions of the DAO in head turn animals. CS electrodes were located in the DLPN or at its border with the middle cerebellar peduncle. Rabbits with US electrodes in the reticular formation or in more caudal portions of the inferior olive that evoked behavioral responses (n=5) failed to condition.

Demonstration of classical conditioning of skeletal muscle responses with direct stimulation of mossy fibers (CS) and climbing fibers (US) provides strong evidence that the cerebellum is critically involved in the acquisition of simple learned motor responses. In addition, the present data support previous theories of cerebellar function (e.g., Albus, Math. Biosci., 1971; Eccles, Brain Res., 1977; Ito, Int. J. Neurol., 1968; Marr, J. Physiol., 1969) which have suggested that motor learning involves interaction of mossy and climbing fiber inputs to the cerebellum.

Supported by NIMH fellowship MH08994 (JES) and ONR contract N00014-83-K-0238, NSF grant BNS-81-17115, and the Sloan Foundation (RFT).

290.7 DEGRADATIVE OPIOID ENZYME ACTIVITY IN PLASMA IS HIGHLY CORRELATED WITH AVOIDANCE PERFORMANCE IN RATS. Joe L. Martinez, Jr., Susan B. Weinberger*, Sandra Morrow*, and Richard C. Dana. Psychology Department, University of California, Berkeley, CA 94720.

We previously reported (Neurosci Abs., 1984, 10, 176) that methylnaloxonium and an antiserum to leu-enkephalin (LE) enhanced acquisition of an active avoidance response, and that LE impaired acquisition of the same response, indicating that peripheral endogenous opioid systems may be involved in the normal machinery of learning. The present study extends these findings by examining the dynamic relationship between opioid metabolism and behavior.

Male (300-450 g) Sprague-Dawley rats were anesthetized (Nembutal) and cannulated in the femoral artery. Two days later the rats were trained in a one way active avoidance task (600 uA footshock). The rats had 10 s to avoid the onset of shock, and 30 s to escape; a total of 8 trials were given. Blood (.5 ml) was withdrawn before the first trial. Enzyme activity was measured by incubating $^3$H-LE in plasma for 1, 2, or 15 min at 37 deg C, followed by the addition of 0.1% TFA in methanol to stop the reaction. Unmetabolized LE was separated from its metabolites by thin layer chromatography using reverse phase KC-18 plates. Metabolism rates were determined with a liquid scintillation counter by comparing the amount of unmetabolized LE to that of its combined metabolites, at each time point. Enzyme activity was found to be correlated with age; there was no correlation between age and latency to escape.

The correlation between the latency to escape on the first trial and the amount of LE metabolism, as corrected for age, was $r(17) = 0.82$, $p = .00001$. The kinetics of LE degradation measured in this study are consistent with those reported by Hambrook et al. (1976; Nature, 262, 782).

The findings demonstrate a strong and direct relationship between a measure of opioid degradation and behavior on the first avoidance trial. These data support the suggestion that endogenous peripheral opioids function to influence complex behavior, and indicate that this relationship can only be revealed by examining a dynamic measure of opioid function. (Supported by ONR N00014-83-K-0408).

290.8 ERROR SIGNALS IN LIMBIC CORTEX (AREA 24) OF MONKEYS DURING MOTOR LEARNING. V.B. Brooks, H. Gemba* and K. Sasaki*. Dept. of Physiology, Univ. of Western Ontario, London, Canada, N6A 5C1; and Inst. for Brain Res., Fac. of Med., Kyoto Univ., Kyoto 606, Japan.

Monkeys learn to perform simple motor tasks in two successive main phases. Progress increases abruptly in transition from the first to the second. During the first phase the subjects gain insight about behavioral task requirements and form stimulus-response associations. During the second, insightful, phase subjects improve skill of motor performance in proportion to their degree of insight, reflected by correct task behavior. Motor skill is improved by rapidly increasing use of 'continuous' movements, distinguished by single-peaked velocity profiles, which are predictively programmed by the cerebro-cerebellar circuit (1,3).

The sequence of two major phases in motor learning has been shown for monkeys reacting to visual cues in order to make horizontal elbow flexions and extensions to turn a handle in a step-tracking task (1,3) and for wrist dorsiflexions to lift a lever (2,4). Behavioral insight follows maximal growth of evoked potentials in prestriate, prefrontal and premotor cortex, preceding correct task performance (2,4). The subsequent sudden increase of insightful learning is accompanied by shortening of reaction times and by growing cerebello-cerebral projection potentials in motor cortex (2,4).

Rostral cingulate cortex (area 24) does not exhibit task-related evoked potentials preceding behaviorally correct movements (5), but we now report that they follow incorrect lever lifts made without reference to the visual task-cues (i.e., after the 900 msec presentation of the visual cues that were repeated on average about every 4 sec). The limbic error signals have been observed during the period of insightful learning ipsi- or contralaterally to the operant arm in two monkeys (M. fuscata). The potentials usually follow incorrect lever lifts within 50 msec. The surface-positive, depth-negative potentials were recorded with electrodes implanted at the cortical surface and at 3 mm depth. Dipole analysis (2,4) suggests their origin to be from cortico-cortical fibers or from thalamo-cortical fibers terminating in deep layers of the cortex. Evoked "error" potentials may represent activation of cingulate "error-recognition" units (6).

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290.9 LEARNING-MEMORY IMPAIRMENTS IN RATS AFTER UNILATERAL KAINATE LESIONS OF THE MEYNERT'S NUCLEUS. A.C.Rossi M.A.Cervini* and M.Buonamici*. Biological R.& D., Farmitalia C. Erba, 20014 Nerviano, Italy.

Some tests aimed at measuring the nociceptive thresholds are also useful tools for the study of learning-memory processes in rats (e.g. "threshold conditioned escape response": Giurgea C., et al., 11th C.I.N.P. Congress, Vienna, Abstracts, 248, 1978). The present experiments were carried out on male Sprague-Dawley rats weighing 250-275 g. 10 animals were normal controls (C) and other two groups of 10 each were respectively sham-lesioned (S) or unilaterally lesioned (L) with kainic acid in the Meynert's nucleus (Johnston M.V. et al. Exp. Brain Res., 43:159, 1981). From the 3rd day after treatments all the animals randomly underwent to repeated measurement of their nociceptive thresholds by 4 different tests: hot plate, 56°C (HP), tail pinch (TP), tail flick (TF) and paw pressure (RS). No significant differences among the three experimental groups did appear in nociceptive thresholds in three (TP, TF and RS) of the tests used from the 3rd up to the 100th day after treatments. In the context of the HP test, repeated every day, mean nociceptive thresholds of L animals remained almost constant in time, whereas thresholds of S and C animals were progressively reduced. The correct "learning" by S and C animals was further demonstrated placing them on the HP apparatus without heathing the plate: S and C animals showed "retention" jumping away for several days. In the same situation, L animals did not respond at all, showing no "retention", i.e. impairment of learning and memory, in the absence of detectable motor defects as assessed by the rotarod test. It is concluded that this kind of lesion of the Meynert's nucleus in rats does not affect nociceptive thresholds but does impair learning-memory processes as measured by "threshold conditioned escape response" methods.

290.10 REDUCED AFTERHYPERPOLARIZATION AND RAPID ACTIVATION OF CORTICAL CELLS PRODUCED BY ELECTRICAL STIMULATION OF HYPOTHALAMUS IN MONKEY AND CAT. S. Aou*, C.D. Woody, C.D. Chapman*, Y. Oomura and H. Nishino* (SPON: B. Swartz). Depts. of Anatomy and Psychiatry, UCLA Med. Ctr., Los Angeles, CA 90024 and National Inst. Physiol. Sci., Okazaki 444, JAPAN.

Previous studies have shown that short latency activation (<20 ms) of cells of the motor cortex can be elicited by electrical stimulation of the lateral hypothalamus. Such stimulation can help accelerate rates of conditioning in cats and can be used as an operant reinforcer in cats and monkeys (Woody, C.D. et al. J. Neurophysiol., 49: 780, 1983; Aou S. et al. Soc. Neurosci. Abstr. 10: 312, 1984). The present study shows that lateral hypothalamic stimulation can also elicit very short latency (<1 ms) unit activity as well as a reduction of afterhyperpolarization (AHP) in cortical cells of both species.

Lateral hypothalamic stimulation (A: 18-20, L: 2-4, H: 1-3) evoked action potentials with latencies <1 ms in 38 of 125 motor cortex neurons in monkeys (macaca fuscata). Comparably short latencies of activation were found in a smaller proportion of motor cortex cells in cats. Some responses followed stimulation at 300 Hz with fixed latency and met collision tests. A direct cortico-hypothalamic projection has been reported in the monkey (Nauta, W.H.J., Acta Neurobiol. Exp. 32: 125, 1972).

Following electrical stimulation of the lateral hypothalamus with a 4 or 5 pulse train (100-500 μs, 50 Hz, 0.5-1.5 mA, bipolar), 14 of 23 cells in monkeys showed a reduction in both amplitude and duration of the AHP with little or no accompanying change in levels of spontaneous resting potential. The effect began 15 to 70 ms after stimulation and persisted for 50 to 300 ms after stimulation. Sometimes, a decrease in the threshold level for spike generation accompanied the AHP reduction. This phenomenon could also be observed in neurons of the motor cortex of awake cats together with increases in input resistance.

These studies provide evidence in two different mammalian species for shared commonalities in hypothalamo-cortical interactions which are of potential significance to development of learned behavior. (Supported in part by AFOSR and NICHHD.)

290.11 THE ACOUSTIC PINNA REFLEX : A MODEL SYSTEM FOR THE BEHAVIORAL AND ELECTROPHYSIOLOGICAL ANALYSIS OF DRUG EFFECTS, HABITUATION, PREPULSE INHIBITION, AND FEAR CONDITIONING IN RATS. J.V.Cassella and M.Davis. Dept. of Psychiatry, Yale Univ. Sch. of Med., New Haven, CT 06508.

A reliable component of the acoustic startle response in animals is a flexion of the ears, the pinna reflex. One goal of the present investigation was to develop a preparation and apparatus suitable for analyzing the pinna reflex in the awake rat. The second goal was to examine the pinna reflex under behavioral and pharmacological conditions known to affect other response systems like the whole-body startle reflex and determine if the pinna response is a valid model for analyzing behavioral plasticity. The third goal was to assess the viability of conducting single-unit analysis of mechanisms mediating plasticity of the acoustically-elicited pinna response in these awake rats.

For behavioral testing, male albino rats were first spinally transected under halothane anesthesia and then implanted with a stainless steel bolt which was attached to the skull. In other cases, the spinal transection procedure was replaced by epidural anesthesia of the spinal cord via chronically implanted PE-10 tubing in the epidural space at T-2. Infusion of 10% procaine produces a reversible mid- and hind-body paralysis thus allowing for pinna measurement and unit recording in awake, restrained animals. Awake animals were subsequently mounted in a modified Kopf stereotaxic instrument using the implanted bolt. Acoustically-elicited pinna movement was measured with a photocell device. At rest the pinna interrupted a light beam. However, movement of the pinna in response to an acoustic stimulus activated a phototransistor which produced a graded output proportional to the magnitude of pinna movement.

The amplitude of the pinna reflex was directly related to stimulus intensity, exhibited short-term habituation, pre-pulse inhibition, and enhancement by prior fear conditioning. Pinna response amplitude was increased following systemic administration of strychnine and decreased following clonidine. It is concluded that this preparation and response system are valid for studying various forms of behavioral modification.

Other investigators using anatomical tracing techniques have indicated that pinna muscles are innervated by the medial subdivision of the facial motor nucleus (FMN). Electrophysiological investigation of the FMN in the present study revealed that cells in the medial division are accessible to single-unit analysis in this awake preparation and respond with a latency of less than 6 msec to an acoustic startle stimulus. Currently we are analyzing single-unit activity of the FMN during changes in the pinna response in the awake, spinally anesthetized rat.

290.12 ASSESSING THE INHIBITORY LONG-TERM HABITUATION MECHANISM USING THE TIME COURSE OF THE BEHAVIORAL ACOUSTIC STARTLE RESPONSE IN THE RAT. Wesley P. Jordan, Psychology, St. Mary's College of Maryland.

Auditory stimuli to a rat of sufficient intensity to provoke a startle reflex produces two independent types of response habituation. A transient, short-term habituation is mediated by neural mechanisms within the stimulus-response (S-R) pathway for the reflex in the caudal brainstem. Superimposed upon this short-term mechanism is a relatively permanent, long-term habituation. Long-term habituation requires inhibitory neural mechanisms extrinsic to the S-R pathway of the response. Lesions to either the midbrain reticular formation (MRF) or to the vermis of the cerebellum severely attenutes or abolishes habituation over days without altering short-term habituation. This long-term habituation mechanism actively inhibits the S-R pathway on each stimulus presentation.

The extra synapses necessary for the extrinsic habituation mechanism requires that the influence of these elements on the response occurs shortly after the stimulus has elicited the reflex. By sampling rapidly with a microcomputer the activity of the stabilimeter used to measure the startle response, it is possible to chart the time course of the response.

On the initial stimulus presentation, the amplitude of the response peaked within the first 100ms from stimulus onset and then declined to baseline within approximately 300ms. With habituation, the amplitude of the peak of the startle response declined and the slope of the response curve from the peak back to baseline became more negative (i.e., became steeper). There was no systematic change in the latency of the peak response during habituation. Of particular interest was a suggestion that the steep slope of the response from peak to baseline was due to the arrival of the inhibitory influence from the extrinsic mechanism. This was suggested by two observations. First, the responsiveness approximately 200ms after stimulus onset was below the baseline of activity seen several hundred milliseconds later and occured only after habituation. Second, animals with lesions in the MRF failed to show this change in slope.

These results must be interpreted with caution until the dynamics of the test apparatus's response can be evaluated fully. A more direct approach that measures the EMG response is in progress.

290.13 DENDRITIC SPINES AND ASSOCIATIVE SYNAPTIC PLASTICITY.
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Previous theoretical studies (Perkel and Perkel, 1985; Miller et al., 1985) have shown that active membrane in spine heads can lead to amplification of the postsynaptic potential in the dendrite, as compared with synaptic placement on a passive spine head or on the dendritic shaft. Other studies (Shepherd et al., 1985) have shown that, in a population of spines having active membrane, stimulation of a sufficient number of synapses produces a cooperative effect, in which large-amplitude depolarizations are produced in groups of neighboring spines, which in turn can propagate nearly non-decrementally along the dendrite, for as long as there is a sufficient density of active spines.

A class of theories on the cellular basis of plasticity (G. S. Lynch et al., 1985) proposes that as a consequence of repeated stimulation of synapses on dendritic spines, the synaptic efficacy is enhanced; in the particular case of long-term potentiation (LTP), the mechanism is thought to be an increase in the number of effective receptors for neurotransmitter, the increase being mediated through calcium-ion accumulation in the spine head. LTP is increased heterosynaptically, i.e., when several synapses are activated concurrently. However, if the cooperative effect of active spines were operative, the large depolarizations in a widespread population of spine heads would be expected to result in high levels of calcium ion in most such spine heads, and the consequent enhancement of synaptic efficacy would be too widespread and nonspecific to serve as a plausible learning mechanism.

Accordingly, we have explored the effects of transmitter-actuated, voltage-dependent calcium-ion channels, alone and in combination with the usual voltage-dependent channels. When the degree of voltage dependence in transmitter-actuated channels is sufficient, both signal amplification and cooperative effects are produced; however, the required degree of voltage dependence is higher than is usually attributed to depolarizing synaptic conductances.

With more modest degrees of voltage dependence in these channels, suffient amplification is provided through additional inward-current channels (not activated by transmitter). Cooperative effects may then be effectively limited to those spines under concurrent synaptic activation. As a result, calcium-mediated subsynaptic modification is confined to those synapses that are repeatedly and simultaneously activated; neither the large depolarizations nor the synaptic enhancement spread beyond the appropriate synapses. Such combinations of channels are suggested as possible substrates for LTP and associative learning in such systems as the hippocampus.

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## FUNCTIONS OF GLIA III

291.1 ASTROCYTIC CELL CLONES FROM MOUSE CEREBELLA : EFFECTS ON SURVIVAL AND DIFFERENTIATION OF EMBRYONIC NEURONES.
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To investigate the role of astrocytes on the survival and differentiation of neurones, we have used astrocytic clonal cell lines which we have derived from 8 day post-natal mouse cerebellum and might be the in vitro counterparts of the fibrous and velamentous astrocytes and of the Golgi epithelial cells (Alliot, F. and Pessac, B., *Brain Res.*, 306:283, 1984). Single cell suspensions from cerebellum of 15 day embryonic mice were seeded upon confluent monolayers of each of the astrocytic cell clones. About 80 % of the embryonic neurones, identified by tetanus toxin binding, adhered within 3 hours. The astrocytic clones had distinct effects on the survival of these neurones. On the Golgi epithelial cells and the velamentous astrocytes, the number of neurones remained constant or increased ; in contrast, only 10-20% of the neurones that had adhered to the fibrous astrocytes were present after 5 days of coculture. This "toxic" effect could be mimicked by conditioned media of fibrous astrocytes.

The differentiation pattern of the neurones varied markedly between the cocultures with the 3 types of astrocytic cell clones. After 48 hours, the majority of neurones cultured on Golgi epithelial astrocytes appeared highly branched often with a spiderweb morphology, while many neurones extended a network of recurrent processes on the velamentous astrocytes. Most neurones present on the fibrous astrocytes had few long unbranched processes. These differences in neuronal morphology were more pronounced in cocultures maintained up to 12 days. In addition these morphologically differentiated neurones acquired distinctive markers ; specific anti-GABA antisera (given by A. Towbie and M. Geffard) labeled 50% and 25% of the neurones maintained respectively on the velamentous or on the Golgi astrocytes and monoclonal antibodies specific for cerebellar neurones bound with a distinct pattern to the embryonic neurones cocultured with the astrocytic clones.

Taken together these data indicate that each astrocytic type has a distinct effect on the survival of neurones and induces a distinct differentiation pattern in embryonic cerebellum neurones.

291.2 NEURON-GLIAL CONTACTS INDUCE ASTROGLIAL DIFFERENTIATION.
M.E. Hatten, M. Woods*, J. Sanchez*, C.A. Mason and R.K.H. Liem. Dept. Pharmacology, NYU Sch. of Med., New York, NY 10016.

In vitro studies on purified mouse cerebellar granule neurons and astroglia have revealed that neurons induce the expression of complex astroglial shapes needed for neuronal migration and regulate the rate of glial proliferation. Here, we have used the technique of recombining purified cerebellar granule neurons and astroglia in vitro to measure the time course of the effects of neurons on astroglia and analyze the importance of cell-cell contacts for astroglial differentiation.

The time course of the effects of neurons on glial form and cell division is rapid. Neurons bind to glia within minutes and $^{3}$H-thymidine incorporation drops several thousand-fold within 3-6h. This result depends on the number of neurons added, with a threshold at one neuron per astroglial cell. Also within 3-6h of neuronal binding to the glia, the amount of glial filament protein, measured by immunocytochemistry, increases dramatically, the glial cytoskeleton reorganizes and glial form changes from an undifferentiated, flat shape into highly elongated shapes. Over the next 36h, the astroglia transit through a series of forms from simple, elongated shapes to the complex shapes commonly seen in microcultures of postnatal cerebellar cells.

To analyze whether the effects of neurons on astroglial morphology and differentiation are mediated by trophic factors or contact interactions, we tested conditioned medium, fixed neurons and neuronal membranes. Medium conditioned by neurons alone or by both neurons and astroglia does not induce astroglial differentiation. Similarly, the co-culture of neurons and astroglia in separate wells with co-mingled medium does not effect glial growth or shape.

The effects of fixed neurons and neuronal membranes are similar to results seen with intact, viable granule neurons. Adding fixed neurons (0.5 percent paraformaldehyde) or neuronal membranes to purified glia inhibits astroglial $^{3}$H-thymidine incorporation and induces glial morphological differentiation. The major difference between intact cells and fixed cells is the duration of the effect. Intact neurons regulate glial proliferation and shape for periods as long as a week, fixed cells for 24-48h only.

These experiments suggest that cell-cell contacts between neurons and astroglia regulate astroglial cell division and differentiation. Supported by NIH grants NS 15429 and NS 21097 (MEH) NS 16951 (RKHL) and NS 15182 (CAM), The Alfred P. Sloan Foundation and the Irma T. Hirschl Trust.

291.3 ASTROCYTES IN THE DORSAL COLUMNS OF THE DEVELOPING SPINAL CORD. T. J. Sims,[1] S. A. Gilmore,[1] J. S. Kenney*[2] and S. G. Waxman[2]
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Astrocytes and their related cells, the radial glia, have been implicated in the guidance of axons during normal development. This issue, however, remains to be settled. In this study astrocytes in the midline of the dorsal spinal cord in normal and irradiated rats were examined postnataly to assess their involvement with developing axons of the dorsal columns. For the irradiation procedures a beam of x-rays (4000R) was localized to a 5 mm segment of lumbosacral spinal cord in 3-day-old rats. Normal and irradiated lumbosacral spinal cords were examined by immunocytochemical staining against GFAP and by electron microscopy. Two antibodies directed against GFAP were used; one was a polyclonal anti-GFAP ( source L. Eng ) and the other a monoclonal against both GFAP and vimentin ( source J. Kenney and T.Sims ). At 7 days postnatal the first cells to exhibit immunoreactivity in the dorsal columns had radial processes in the midline oriented toward the surface of the cord. At 14 days many positively stained astrocytes were observed in the dorsal half of the columns and were contrasted by the lack of staining in the cells of the dorsal funiculus adjacent to the dorsal columns. Ultrastructural identification of 9 nm filament-containing cells in the midline at 7 days confirmed that these cells were radial glia. In the normal and irradiated spinal cord the processes of the radial glia terminate at the cord surface, where they form, in part, the glia limitans. At 7 and to a greater degree at 14 days, radial glia project finger-like processes around bundles of axons. In the irradiated cord at 14 days, ectopic axons were seen on the dorsal surface of the glia limitans outside the confinement of the cord. In this situation radial glia extend finger-like projections to contact or surround these ectopic axons. At 25 days, ectopic axons were no longer seen on the surface of the cord. These findings strongly suggest that GFAP positive radial glia play an active (and a corrective) role in the maintenance of spinal cord architecture. Supported by NIH NS-04761 and NS-15320.

291.4 OUTGROWTH OF ASTROGLIAL PROCESSES IN DISSOCIATED CEREBELLAR CULTURES. C.A. Mason and M.E. Hatten. Dept. Pharmacology, N.Y.U. School of Medicine, New York, NY 10016.

Little is known about the induction and elongation of the processes of astroglia. With the in vitro model system developed in this laboratory, we have asked how neurons associate with glia during outgrowth and compared glial process outgrowth to neuronal process outgrowth. Cultures of dissociated mouse cerebellum at postnatal day 4-7 were observed 1, 3, 5, 8, and 24 hrs after plating, with a combination of immunocytochemistry with antisera to glial filament protein, electron microscopy, and video time-lapse microscopy. The forms, activity, and corresponding cytology of glial processes, particularly the stellate astroglial forms, and the cell-cell contacts of neurons with glia during the various phases of outgrowth were studied. Three phases of glial process outgrowth and neuron association occur: 1). 1 hr - rounded neurons and glia fall to the surface of the dish. Astroglia contain scattered glial filaments in their soma, their cell surface is highly ruffled, and extension and withdrawal of short filopodia take place. 2). 3-8 hrs - rapid extension of glial processes. As glial processes originate on the soma, filopodial extension ceases on that aspect of the soma. The growing glial processes end in a growth cone that generally has the motile behavior and appendages of neuritic growth cones (lamellopodia, filopodia). In contrast to neurites, however, the glial growth cone is the leading edge of a thick paddle of cytoplasm, rather than a thin neurite, and glial filaments extend from the process well into the growth cone. The thick glial arms do not branch extensively, and they reach their final length of 50-70 μm during this period. 3). 5-24 hrs - increasing association of neurons with glia. When first plated, neurons and glia are randomly arranged. By 5 hrs, neurons begin to preferentially associate with glia, and veil-like structures and filopodia extend from glial arms near the soma to encompass groups of neurons. By 24 hrs, at low power, islands of cells with neurons positioned on glia are well-formed. These experiments suggest tht glial process outgrowth results in specific glial forms and that neurons might play a role in this process. (Supported by NIH Grant NS 15182, and The Alfred P. Sloan and Irma T. Hirschl foundations).

291.5 ASTROCYTES OF THE CENTRAL NERVOUS SYSTEM (CNS) AND NON-MYELINATING GLIA OF THE PERIPHERAL NERVOUS SYSTEM (PNS) ARE ASSOCIATED WITH APOLIPOPROTEIN E. J.K. Boyles*, R.E. Pitas* and R.W. Mahley* (SPON: R.M. Nitkin). Gladstone Foundation Laboratories, University of California, San Francisco, CA 94140.

The plasma protein apolipoprotein E (apo-E) ($M_r$ = 35,000) is an important determinant of lipid transport and lipoprotein metabolism in mammals. Although a major source of apo-E production is the liver, recent work has identified apo-E mRNA in high concentration within the brains of rats, marmosets, and man (Elshourbagy et al., **PNAS**, 82:203, 1985). In the present study, apo-E has been identified with specific cells of the CNS and PNS by light and electron microscopic immunocytochemistry using cryostat sections of formaldehyde-fixed tissue.

All astrocytes from each of the major subdivisions of the CNS, including specialized astrocytic cells — Bergmann glia, tanycytes, pituicytes, and Müller cells — contained significant concentrations of apo-E. Neurons, oligodendroglia, microglia, ependymal cells, and choroidal cells did not. In dual labeling experiments, apo-E was expressed with equal intensity by all astrocytic glia, whereas GFAP was variable in its expression. Apolipoprotein E was present not only in the perinuclear region of astrocytic cells, but also in those cell processes ending on basement membranes at either the pial surface or along blood vessels. Extracellular apo-E was present along many of these same surfaces, suggesting that apo-E is secreted by astrocytic cells. This impression was confirmed by electron microscopic immunocytochemical studies: apo-E could be identified in the Golgi apparatus, which is a secretory organelle. In culture, astrocytes continued to secrete apo-E. The apo-E extracted from rat brain was similar to that secreted by astrocytes in culture but differed from plasma apo-E. Brain apo-E had a slightly higher apparent molecular weight ($M_r$ = 36,000) and was composed of more acidic isoforms.

In the PNS, apo-E was found by light level immunocytochemistry to be present within the perinuclear region of those glia that surround neurons (satellite cells of the cervical dorsal root ganglia and superior cervical sympathetic ganglion as well as the enteric glia of the intestinal ganglia). Apolipoprotein E was also present, but to a lesser extent, within the non-myelinating Schwann cells but not within the myelinating Schwann cells of peripheral nerves (cervical sympathetic trunk, brachial plexus, sciatic nerve, and cervical spinal nerves).

The secretion of apo-E by support cells of both the CNS and PNS suggests that apo-E has an important, previously unsuspected role in the physiology of nervous tissue. In addition, apo-E secretion promises to be a valuable marker for cells of astrocytic lineage in the CNS and perhaps of non-myelinating glia in the PNS.

291.6 MODULATION OF ASTROCYTE RESPONSES BY EPIDERMAL GROWTH FACTOR, FIBROBLAST GROWTH FACTOR, AND PLATELET-DERIVED GROWTH FACTOR. K. R. Huff* and W. Schreier*. (SPON:L.Erinoff) Neurology Research Lab, Children's Hosp. L.A., Univ.Southern Calif. Sch.of Med., Los Angeles, CA 90054.

There must be controlling signals for the proliferation of astrocytes in the developing brain and in the reactive gliosis response. Epidermal Growth Factor (EGF), Fibroblast Growth Factor (FGF), and Platelet-Derived Growth Factor (PDGF) are found in the nervous system but much remains to be known about the nature of the astrocyte response to these signals. For example it is not known whether these growth factors may modulate each other's effect on astrocyte behavior.

Primary astrocyte cultures have been purified from the cerebral cortex of 1 day rat pups and maintained for several months in media containing fetal calf serum (FCS) or several weeks in serum free media. The cells have a flat polyhedral undifferentiated morphology but contain bundles of cytoplasmic filaments which stain intensely for glial fibrillary acidic protein. EGF stimulated a maximal increase in cell number at 10 ng/ml and an increase in thymidine incorporation for DNA synthesis at 2 ng/ml at 24 hours. FGF also mildly stimulated thymidine incorporation. The rate of leucine incorporation into protein was not stimulated by EGF, although it did not decline and the cell total protein increased with higher EGF concentration. The mitogenic effects and increase in protein by EGF treatment was not as great as with addition of FCS however. EGF produced an absolute increase in ornithine decarboxylase (ODC) activity, a marker for cell trophic responses, at 2 and 4 hours.

Pretreatment of the cells by FGF 50 ng/ml for 18 hours reduced both the EGF-induced increase in cell number and thymidine incorporation rate into DNA. PDGF pretreatment 0.5 unit/ml for 18 hours also reduced the EGF-induced increase in thymidine incorporation at lower EGF concentrations of 0.5-1.0 ng/ml but not higher. Dibutyryl cyclic AMP (dBcAMP) 1mM pretreatment did not have this effect. The pretreatments did not effect EGF-induced leucine incorporation. Although the growth factors increased the protein content of the cultures slightly, pretreatments with FGF and PDGF but not dBcAMP consistently reduced the thymidine incorporation stimulation produced by FCS treatment.

These data support the conclusion that EGF has a definite mitogenic influence on astrocytes which is dose and time dependent. It may have a maintainence influence on protein catabolism and stimulate an ODC mediated trophic response. Although FGF was mitogenic to a lesser extent, pretreatments by both FGF and PDGF reduced the mitogenic effect of both EGF and serum. Possibly this pretreatment effect is through heterologous down regulation of EGF receptors. These findings point to a modulation or turning off effect through growth factor interaction of proliferative signals to the astrocyte.

291.7 **ANTEROGRADE SPREAD OF SCHWANN-CELL MITOSIS IN WALLERIAN DEGENERATION** A. L. Oaklander and P. S. Spencer. Institute of Neurotoxicology and Departments of Neuroscience and Pathology, Albert Einstein College of Medicine, Bronx, N.Y. 10461

Neuron-glia communication is required for events such as nerve development, myelination, degeneration and regeneration, but little is known about how it occurs. We are studying axon-Schwann cell relations during Wallerian degeneration (W.D.) in the peripheral nervous system. Transection of a peripheral nerve triggers a series of phenotypic alterations in Schwann cells distal to the injury, of which mitosis is one of the earliest and best characterized events. This study examines the spatio-temporal spread of premitotic DNA synthesis along transected peripheral nerves.

Sciatic nerves of anesthetized adult cats were transected at the notch. Animals were housed at stable temperature for 2.5, 3, 3.25, 3.5, or 4 days following surgery. Three cats (6 sciatic nerves) were used at each timepoint. Operated animals were reanesthetized and distal stumps excised from the transection site to the level of the ankle. Nerves were sliced into 2-mm segments, desheathed, and incubated for 2 h at 37°C in tissue culture medium M199 containing $^{3}$H-thymidine (85-90 Ci/mmol). Following incubation, nerve pieces were rinsed in saline, then homogenized and washed 3 times in 10% trichloroacetic acid containing 0.1% thymidine. Remaining insoluble material was dissolved in 1N sodium hydroxide. Incorporation of $^{3}$H-thymidine was measured by scintillation counting, protein by the Lowry method, and results expressed as femtomoles $^{3}$H-thymidine incorporated/microgram protein.

At 2.5 and 3 days post-transection, $^{3}$H-thymidine incorporation was enhanced only in the most proximal portion of distal stumps. In contrast, by 4 days, incorporation was elevated along the entire length (14-18 cm) of excised nerve. Previous autoradiographic studies have shown that Schwann cells comprise approximately 80% of $^{3}$H-thymidine-labelled nuclei in 4-day distal stumps. At 3.25 days, the elevation in $^{3}$H-thymidine incorporation had advanced to approximately 11 cm distal to the transection site, and at 3.5 days the front had progressed virtually to the end of the nerves studied. Sham-operated nerves exhibited extremely low baseline levels of incorporation of $^{3}$H-thymidine.

These data suggest that Schwann cells close to the site of nerve injury enter premitotic S-phase prior to those located more distally. Following a lag of approximately 3 days, distal Schwann cells enter S-phase within approximately 12 h. S-phase appears to be initiated in a proximo-distal sweep whose rate is comparable to that of fast axonal transport.

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291.8 SERUM ASCORBIC ACID REGULATES MYELIN FORMATION AND BASAL LAMINA ASSEMBLY BY SCHWANN CELLS IN VITRO. C.F. Eldridge*, M.B. Bunge, and R.P. Bunge. Dept. of Anat. and Neurobiol., Washington Univ. Sch. Med., St. Louis, MO, 63110.

Rat Schwann cells co-cultured with sensory neurons in serum-free (N2) medium are viable for many months in vitro but fail to ensheathe or myelinate axons and do not form a basal lamina. Supplementation of N2 medium with either human placental serum plus chick embryo extract (EE) or fetuin plus ascorbic acid has been reported to stimulate Schwann cell myelination and basal lamina assembly (JCB 97:369a,1983; JCB 99:404a,1984). We have made counts of myelin segments in whole mount cultures to study the relative efficacy of these supplements in promoting myelination and have made parallel observations of the deposition of basal lamina components using immunocytochemistry. In agreement with previous reports we found that cultures grown in N2 medium alone lacked both myelin and extracellular basal lamina components (collagen type IV, heparan sulfate proteoglycan) except for a sparse punctate distribution of laminin on the Schwann cell surface. Whereas the addition of fetuin plus ascorbic acid to N2 medium promoted myelin formation (mean number of myelin segments/sq.mm=65, SEM=31, n=5 cultures), the effect was less pronounced and more variable than after the addition of serum plus EE (mean=290, SEM=12, n=5). The serum used could be divided into two categories according to whether or not EE was required for myelin formation. Some lots of serum promoted myelination in the absence of EE; dialysis of such serum abolished its ability to stimulate myelination, and the addition of ascorbic acid to the dialyzed serum restored the ability. Other lots of serum did not promote myelination unless EE was present; the addition of ascorbic acid to such serum (either whole or dialyzed) was sufficient to promote myelination equal to or greater than that achieved after the addition of EE. Myelin formation in N2 medium supplemented with ascorbic acid alone was minimal, indicating a requirement for a non-dialyzable serum component. There was a strict correlation between myelin formation and the deposition of basal lamina components by Schwann cells under all conditions tested. The role played by serum is not understood; ascorbic acid is known to promote the formation of triple-helical collagen molecules, which are not detectable in cultures fed N2 medium alone. Although a direct effect of ascorbic acid on the process of myelination itself has not been ruled out, we believe that an important role of ascorbic acid is to indirectly promote myelin formation by enabling the Schwann cell to surround itself with a basal lamina stabilized by triple-helical collagen.

(Supported by NIH grant NS09923)

291.9 NEURONAL REGULATION OF BASAL LAMINA FORMATION BY SCHWANN CELLS. M.B. Clark*, C.F. Eldridge*, and M.B. Bunge. Dept. Anat. & Neurobiol., Washington Univ. St. Louis, MO 63110.

Earlier studies from this laboratory demonstrated that the presence of nerve cells is required for the formation of a basal lamina on the Schwann cell (SC) surface in tissue culture (Bunge et al., 1982). In these cultures SCs and neurons (Ns) were in contact with each other. The present study investigated whether direct contact between Ns and SCs is required for the generation of basal lamina. SCs on 10 mm coverslips were co-cultured on 37 mm bed cultures of SCs, Ns, or SCs + Ns according to one of four conditions: 1) as SCs in contact with Ns (SCs+Ns) on coverslips placed on beds of SCs in contact with Ns (SCs+Ns/SCs+Ns), 2) as isolated SCs on beds of SCs in contact with Ns (SCs/SCs+Ns), 3) as isolated SCs on beds of Ns (SCs/Ns), and 4) as isolated SCs on beds of SCs (SCs/SCs). Coverslip cells and surrounding bed cells were not in contact. Co-cultures were maintained for 3-4 weeks in medium known to induce myelination (Eldridge et al., this volume). Accumulation of basal lamina components, such as type IV collagen and heparan sulfate proteoglycan, was assessed by light microscopic immunocytochemistry; formation of basal lamina was assessed by electron microscopy.

Our results indicated that contact between Ns and SCs was necessary for the formation of a linear immunostaining pattern typical of well-differentiated basal lamina and for the formation of an organized continuous basal lamina fine structure. Nevertheless, isolated SCs on beds of SCs + Ns (SCs/SCs+Ns), Ns (SCs/Ns) or SCs (SCs/SCs) exhibited some positive staining for basal lamina constituents, although the pattern of this staining was not linear around the isolated coverslip SCs. This indicated that uncontacted SCs could synthesize and secrete at least some basal lamina components. Electron microscopy of these isolated SCs demonstrated that organization into a continuous basal lamina had not occurred. In aggregates of isolated SCs (SCs/Ns, SCs/SCs, or SCs/SCs+Ns), however, basal lamina-like material had accumulated between the clustered SCs, and discontinuous segments of basal lamina sometimes appeared on SC surfaces. Accumulations of basal lamina-like material noted electron microscopically among clustered SCs corresponded to patches of immunostaining at the light microscopic level.

In summary, these studies demonstrate that direct contact between SCs and Ns is not essential for SCs to synthesize and accumulate at least some basal lamina constituents, but contact is required for the development of a well-organized, continuous basal lamina. (Supported by NIH grant NS 09223.)

291.10 MONOCLONAL ANTIBODY RAT-401 IDENTIFIES DEVELOPING SCHWANN CELLS. B. Friedman and S.J. Hockfield. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724

Previous work in this laboratory, generated a monoclonal antibody, Rat-401, that stains embryonic nonneuronal cells in the rat peripheral and central nervous system. The stained nonneuronal cells in the central nervous system become the radial glial cells, as shown by their morphology and by the close temporal correlation of their appearance and disappearance with the period of neuronal cell migration. The fate of the Rat-401 nonneuronal cells in the peripheral nervous system is the focus of the present study, which presents evidence that these cells survive and become differentiated Schwann cells.

Immunoreactivity of Rat-401 with nonneuronal cells was examined in adult and developing peripheral nerve and in mixed Schwann cell-fibroblast cultures prepared from peripheral nerve from rats at postnatal day 2. Rats were transcardially perfused with 4% paraformaldehyde and fixed peripheral nerve was dissected and stored in fixative. Nerve was sectioned at 100 $\mu$m and reacted with Rat-401, followed by antimouse IgG antibody conjugated to horseradish-peroxidase (HRP). The HRP was visualized with diaminobenzidine and the sections were subsequently dehydrated, embedded in plastic and sectioned for light and electron microscopy. Schwann cells and fibroblasts grown on glass coverslips in tissue culture were tested for Rat-401 immunoreactivity after removal of the media and fixation with 4% paraformaldehyde. Immunostained cultures were dehydrated and mounted on glass slides with permount.

Rat-401 recognizes Schwann cells that have ensheathed axons in early postnatal peripheral nerve. At the light microscopic level, the staining appears to be distributed throughout the cell cytoplasm. Rat-401 recognizes morphologically differentiated Schwann cells in mature nerve. At the electron microscopic level, the Schwann cell cytoplasm is immunoreactive but the subjacent myelin sheath is unstained. Peripheral nerve endoneurium is also not stained, an indication that fibroblasts are not recognized by Rat-401. This has been corroborated by Rat-401 immunostaining of nonneuronal cell cultures. Schwann cells, identified by their spindle shapes, are intensely immunostained while fibroblasts, identified by their irregular flattened profiles, fail to be recognized by Rat-401.

These results indicate that Rat-401 stains Schwann cell cytoplasm in differentiating and mature Schwann cells as well as Schwann cells deprived of axonal contact in cell culture. This suggests that the embryonic nonneuronal cells present in peripheral nerve prior to axon outgrowth (Hockfield and McKay, 1985) are early Schwann cells and may play a role in the support of growing axons.

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291.11 HIGH MOLECULAR WEIGHT GLYCOPROTEINS RELATED TO MAG ARE FOUND IN ACTIVELY MYELINATING OLIGODENDROCYTES BUT NOT IN MYELIN.
J.R. Gulcher,* L.S. Marton,* and K. Stefansson* (SPON: B.G.Arnason) Dept. of Neurology, U. of Chicago, Chicago, IL 60637

Myelin-associated glycoprotein (MAG) is a minor constituent of CNS myelin. Previous work on developing human CNS has demonstrated the existence of several high molecular weight glycoproteins (130 to 250kd) that share at least three epitopes with MAG (Marton and Stefansson, JCB, 1984, 99, 1642).

These glycoproteins appear before MAG and are undetectable in the mature adult. More specifically, these proteins seem to be present only in areas of the CNS undergoing active myelination.

We have isolated one of these polypeptides (130kd) in the following manner:

1) Centrifugation (90,000g) of human fetal brain homogenate in Tris-saline buffer.
2) The supernatant was brought up to 30% Ammonium sulfate at pH 7.0.
3) After centrifugation (10,000g) the supernatant was brought up to 40% AS.
4) The pellet (10,000g) was resuspended and put over a MonoQ anion exchange column (Pharmacia).

The 130kd polypeptide is 15-20% carbohydrate based on deglycosylation with Endoglycosidase F.

Affinity purified polyclonal antibodies specific for the 130kd protein that do not bind to MAG were used for immunohistochemical staining of adult and fetal (30 weeks) spinal cord. We found:

1) No staining of myelin in either the adult or fetus.
2) No staining of cells in adult.
3) Strong staining of cells that appear to be actively myelinating oligodendrocytes in the fetus.

These preliminary results indicate to us that this glycoprotein may have an important but as yet, unknown role in myelination.

291.12 THE DEVELOPMENT OF TRANSFERRIN-POSITIVE CELLS IN THE RAT CNS
J.R. Connor and R.E. Fine* Lab. of CNS Injury and Regeneration, Veterans Administration Medical Center, Washington DC. 20422, Dept of Physiology, George Washington University School of Medicine, Washington DC and Dept. of Biochemistry, Boston University School of Medicine, Boston MA, 02118

Transferrin is a plasma protein whose major function is iron transport and mobilization. In an earlier study we demonstrated that Tf is present in oligodendrocytes and endothelial cells in the adult rat brain. The purpose of this experiment is to describe the development of Tf positive cells in the rat CNS.

Rat pups were perfused with 4% paraformaldehyde and the tissue to be studied was removed at cut on a cyrostat at 16-32 um thickness. Thus far, animals at 7(n=2),8(n=1), 20(n=2), 23(n=1) and 30(n=2) post natal days have been examined. Antiserum to rat Tf prepared in rabbits was utilized and the reaction sites visualized using either PAP or ABC. In either case, 3'3' diaminobenzidine was the chromogen.The adult pattern of Tf-immunoreactivity is seen in the cerebral cortex, corpus callosum, corpus striatum and spinal cord at 30 days of age. Oligodendrocytes are Tf positive and the reaction product is confined to the soma in a "cap-like" fashion. Very few processes of oligodendrocytes are Tf-positive except for those in the corpus striatum and the white matter of the spinal cord.

At 20 days after birth, many Tf positive oligodendrocytes are observed throughout the CNS areas studied and the density and distribution pattern are similar to that observed at 30 days. However, in addition to oligodendrocytes in the spinal cord some anterior motor horn neurons are Tf-positive in the 20 postnatal day group.

In the 7 or 8 day old CNS tissue, very little Tf-immunoreactivity is observed. The greatest density of Tf positive cells in this age group is in the corpus striatum.

The function of Tf in the CNS is still speculative. The development pattern suggests: 1) The storage of Tf in oligodendrocytes occurs or increases to detectable levels after formation of the blood-brain-barrier. The formation of the blood-brain-barrier apparently initiates the requirement for Tf storage because Tf is no longer immediately accessible to the CNS. 2) Tf-Positive cells increase in number at the beginning of myelinogenesis, 3) Tf-immunoreactivity follows the pattern described for carbonic anhydrase labelling of oligodendrocytes.The bicarbonate ion is necessary for Tf to bind iron. No data on the development of iron in the CNS are available. Our findings suggest that Tf is important in myelination and is stored in the mature nervous system.

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## MORPHOGENESIS AND PATTERN FORMATION II

292.1 AN ATLAS OF THE DEVELOPING MOUSE BRAIN.
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This study describes the histological features of the mouse brain during development from embryonic day 10 through adulthood. The atlas will allow identification of both the location and size of particular structures and also the timing of specific developmental events.

Timed matings (within 12 hours) were made between normal C57Bl/6J mice (Jackson), and embryos were removed at daily intervals from the 10th embryonic day through birth (generally at day 19) and for postnatal days 0 (day of birth) through 21, as well as for adults. Embryonic brains were fixed by immersion; older postnatal and adult brains were fixed by cardiac perfusion. 4% paraformaldehyde or 4% paraformaldehyde with 0.5-1.0% glutaraldehyde in a phosphate buffer was used as the fixative. Following dehydration and paraffin embedding, serial sections were made of specimens from each day in both coronal and sagittal planes. Adjacent sections in each series were stained for cell bodies or for fibers so that the growth of nuclei and fiber tracts could be followed.

This study will provide the basis for quantitative morphological investigations on the growth rates of various brain structures. In addition, the atlas will provide valuable information to cellular neurobiologists who wish to determine the optimum locations and time periods to remove portions of the brain for study of cell-cell interactions in vitro.

292.2 PRENATAL TELENCEPHALIC DEVELOPMENT IN THE PRECOCIAL MOUSE, ACOMYS CAHIRINUS.
Peter C. Brunjes, Dept. of Psychology. University of Virginia, Charlottesville, VA 22901

Acomys cahirinus (the "spiny mouse") is born after a 38 day gestation period fully furred, with ears and eyes open and with sophisticated locomotor capabilities. Acomys is a member of the same subfamily (muridae) as the laboratory rat and mouse, which are both born in a very immature state. This fortuitous circumstance has allowed us to pursue an in-depth comparison of rates and patterns of brain growth between closely-related altricial and precocial species. In studies of the postnatal development the olfactory bulb (Dev Brain Res 8,1983,335), hippocampal formation (Brain Behav Evol 24,1984,58) and area 17 of visual cortex (Dev Brain Res in press) we have reported that 1) each of the three regions undergoes a different time course of postnatal growth, suggesting regional control of maturation and 2) two out of the three regions show quite different patterns of postnatal brain development when Acomys is compared to mice or rats of the same post-conception age, suggesting that regional patterns of brain development vary between species and that Acomys is not merely a mouse or rat born late. The present research is an examination of prenatal development in order to have a fuller picture of brain growth in Acomys. Fetuses were obtained every other day throughout the last two-thirds of gestation, brains embedded in either GMA or paraffin and coronal and sagittal sections stained with H&E or toluidine blue. A similar set of sections from rats were also prepared for comparison. At the earliest times examined, (day E 14) the two species seemed to be in a rather similar ontogenetic state. However, by the time of birth in the rat (E 22-23) Acomys was approximately 4 days less mature. For example, the E 22 Acomys olfactory bulb contains a widely patent ventricle, and the only visible layer is that of the mitral cells. The same-aged rat or mouse has developed at least the rudiments of all bulb layers. The neocortex of the E 22-24 Acomys exhibits a dense cortical plate with the first layers barely differentiated at the ventral margin, a situation much less mature than that found in the altricial species. The lag between the species appears to increase, with Acomys approximately a week less mature than the rat by E 25-27. A prenatal growth spurt occurs in Acomys near the end of the fourth week and continues into the postnatal period. The results indicate 1) the notion that precocial species have long gestation periods so that they can be mature at birth is in error, Acomys "wastes" considerable time in its prenatal development when compared to the mouse or rat, and 2) the protracted mid-gestation of Acomys may allow increased resolution into processes of brain growth.

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292.3 DEVELOPMENTAL PATTERN OF THE CELLS OF ORIGIN OF THE CORTICOSPINAL TRACT OF THE RAT. D. J. Schreyer and E. G. Jones. Dept. Anatomy, University of California at Irvine, Irvine, CA 92717.

We have used the retrograde transport of the fluorescent dye Fast Blue injected specifically at cervical or lumbar segments of the spinal cord to label cortical cells projecting axons to these levels of the cord during the first three weeks of life and during adulthood in the rat. The cortical projection to cervical segments is first established during the first few postnatal days by a circumscribed group of layer V pyramidal cells in the dorsal parietal cortex. The set of cortical cells projecting axons at least as far as the cervical cord then expands widely to include layer V cells occupying frontal, parietal and cingulate cortex in a continuous sheet by the end of the first postnatal week. During the second and third postnatal weeks the set of cells contributing axons to the cervical cord and beyond diminishes such that unlabeled areas appear in lateral frontal and lateral parietal cortex, dorsally at the border of frontal and parietal cortex, and medially in medial frontal and cingulate cortex, according to a complex schedule. At the end of the third postnatal week, the adult pattern of cortical cells projecting to cervical levels and beyond is attained. Major concentrations of corticospinal cells occupy frontal, dorsal parietal and ventrolateral parietal cortex including, but not limited to MI, SI and SII, exclusive of the facial representation.

The cortical projection to lumbar segments is first established at the end of the first postnatal week by a circumscribed group of layer V pyramidal cells in dorsal parietal cortex. In contrast to the projection to cervical segments, the projection to lumbar segments undergoes only modest expansion during the subsequent week. The dorsal parietal cell group then maintains the projection to lumbar segments without diminution throughout the developmental period and into adulthood. The dorsal parietal area containing the projection to lumbar segments is the same area that contains the cells initially projecting to cervical segments and includes the hindlimb representation of SI in the adult.

We conclude that the first cortical axons to advance down the spinal cord are those that will innervate the lumbar segments in the adult. Later addition of axons projecting only as far as cervical levels includes those which will establish a persistent connection from frontal and ventrolateral parietal cortex and others that will shortly be eliminated from lateral and medial frontal, lateral parietal and cingulate cortex. At no stage do we observe a substantial projection to the spinal cord from occipital cortex. Supported by NIH Grant NS 10570.

292.4 EARLY HISTOGENESIS IN THE BASAL FOREBRAIN OF THE RAT EMBRYO: THE BASAL FOREBRAIN CELL COLUMN AND ITS ADULT DERIVATIVES. R. Marchand and C. Blanchet*, Laboratoire de Neurobiologie, Hôpital de l'Enfant-Jésus, Québec Canada G1J 1Z4.

Autoradiographic $^{3}$H-thymidine studies dealing with the timing of genesis of the neurons of both the diencephalon and the telencephalon have shown that several of the most early generated neurons of the forebrain are found in the following cell groups: the bed nucleus of the stria terminalis, the lateral preoptic area, the central, medial and anterior cortical nuclei of the amygdaloid complex, the entopeduncular nucleus, the lateral hypothalamic area, the retrochiasmatic nucleus, the dorsal hypothalamic area and the most medial area of the zona incerta.

To study the histogenesis of these early generated cell groups, a series of 13 precisely dated gestating rats were given a pulse of tritiated thymidine at day 11 and 15 hours of gestation. On each subsequent day, the embryos of one gestating animal were killed and prepared for autoradiography. One rat gave birth and its pups were also killed on different days of postnatal life.

As we progress to younger stages of development, the different cell groups so typically organized in the adult forebrain become less clearly circumscribed. It is the radioactive labeling that allows recognition of the early generated neurons identified in adult forebrain. On day 15 of gestation, the heavily labeled neurons of the lateral hypothalamus, of the bed nucleus of the stria terminalis, of the lateral preoptic area and of the amygdala become organized along the longitudinally oriented fibers of the basal forebrain bundle. The longitudinal slab of heavily labeled neurons thus formed in the mantle of the early forebrain was called the basal forebrain cell column. In day 14 embryos, all the heavily labeled neurons of the forebrain form an unbroken cell column extending caudorostrally from the tuberculum posterius to the optic stalk where it divides in two limbs. The heavily labeled neurons of the ventral limb of both sides of the brain merge on the midline in close relationship with the chiasmatic plate primordium. In later stages of development these neurons form the retrochiasmatic nucleus. The neurons of the dorsal limb extends rostrally as far as the ventricular elevation in the floor of the foramen of Monro.

This study discloses that many early isochronically generated cell groups of the forebrain of the rat originate from an ependymal matrix closely asssociated with the ventral diencephalic sulcus and migrate to the early forebrain mantle to constitute the basal forebrain cell column. In later stages of development, the neurons derived from the basal forebrain cell column begin to break up into a series of more definite nuclei. [Supported by the MRC of Canada and the FRSQ].

292.5 MODULAR ARRANGEMENTS OF NEURONAL PROCESSES IN HUMAN CINGULATE CORTEX. F.M. Benes, C.A. Marotta, R. Majocha* and E.D. Bird. Departments of Psychiatry and Neurology, Harvard Medical School and McLean Hospital, Belmont, MA 02178.

The cerebral cortex is known to have unique cytoarchitectural features that are believed to play an important role in its function as a central integrator. Our basic understanding of cortical cytoarchitecture may continue to expand as a result of recent immunocytochemical techniques which can produce widespread staining of neuron-specific markers such as peptides, transmitters and components of the axonal and dendritic cytoskeleton. Recently, it has been found that robust neuron-specific immunostaining in human post-mortem specimens of anterior cingulate cortex is possible using an antibody raised against the neurofilament 200K subunit (NFP-200). Widespread staining, presumed to occur primarily in axonal cytoskeleton, was observed throughout the cortical matrix of 7 control specimens. This neuron-specific staining appeared to occur preferentially in medium and large calibre axons, since stained processes in the sub-cortical white matter were noted to be continuous with fiber bundles in the overlying cortical matrix. Some bundles travelled in a horizontal direction and followed the natural radial curvature of the gyrus, while others were vertical and projected radially toward the molecular layer in a highly ordered arrangement. The distance between neighboring bundles appeared to be uniform for both the horizontal and vertical arrays and produced the net effect of 25-30 μm box-like compartments or modules throughout layers IIIB through VI. These modules, therefore, produced a columnar appearance in both the vertical and horizontal planes. The space lying between adjacent vertical bundles, where there was an absence of NFP-200 immunostaining, may conceivably correspond to the putative minicolumn, since both have a similar width. The fact that modular columns also occurred in the horizontal axis further suggests that cortical processing, which is generally believed to occur predominantly in the vertical plane, may be two-dimensional. Thus, the module may be important as the smallest unit for integration of horizontal with vertical information. Further studies will seek to determine whether other cortical areas, particularly those in sub-human species, show this modular arrangement of NFP-200 immunostained fibers and whether a dementing illness like Alzheimer's disease may involve a disruption of this orderly arrangement of neuronal processes. Supported by RSDA MH 00423, MH 31154, MH/NS 31862, AG 02126, AG 00084, and a McKnight Foundation Award.

292.6 IMMUNOCYTOCHEMISTRY OF NEUROEPITHELIAL BASAL LAMINA ALTERATIONS IN RAT EMBRYOS WITH GENETIC HYDROCEPHALUS. K.S. O'Shea, C.J. D'Amato, and S.P. Hicks. Dept. of Anatomy & Cell Biology and Dept. of Pathology, Univ. of Michigan Medical School, Ann Arbor, MI 48109.

The neuroepithelial basal lamina (BL) appears to play a crucial role in controlling cell-cell interactions during early CNS development, particularly between neuroepithelium (NE) and underlying mesenchyme. In the current study, the distribution of BL components, laminin and collagen IV, were examined in developing control embryos, and embryos homozygous for a gene which produces prenatal hydrocephalus.

Embryos were removed from pregnant rats on the afternoon of days 12-14 days development (1st day = sperm +), immersion fixed for 1 h at room temperature in 4% paraformaldehyde, 0.2% glutaraldehyde in 0.1 M phosphate buffer. Embryos were then cryoprotected in 20% sucrose, and 8 um frozen sections cut through the cephalic region. Sections were incubated in rabbit anti-laminin antibody (1:50) or rabbit anti-collagen IV antibody (1:100) for 2 hours, followed by goat anti-rabbit IgG-FITC (1:50) for 30 minutes. Additional controls were not exposed to the primary antibody. Sections were then viewed and photographed in a Leitz Orthoplan photomicroscope.

Except in regions of active neural crest cell migration, distribution of laminin and collagen IV immunoreactivity appeared very similar in control embryos; following the smooth uninterrupted contour of the BL. However, in hydrocephalic embryos there were numerous interruptions in BL staining for these components. Collagen IV distribution was especially patchy and unlike controls, there was little collagen IV present in the BL of hydrocephalic embryos on the 12th day, but by the 14th day considerable immunoreactivity was observed. These results suggest a delay in synthesis or deposition of this structural element in the neuroepithelial basal lamina of hydrocephalic embryos.

Current investigations are in progress to extend these observations to the ultrastructural level. Supported by NIH grants NS-19825 and NS-21108.

292.7 ASYMMETRIC DEVELOPMENT OF THE HIPPOCAMPAL REGION IN THE SHAKER SHORT-TAIL (sst) MUTANT MOUSE. R.S. Nowakowski and D. Wahlsten. Dept. of Anat., U. Miss. Med. Ctr., Jackson, MS 39216 and Dept. of Psych., Univ. of Waterloo, Waterloo, Ont., Canada N2L 3G1.

Shaker short-tail (sst) is an autosomal recessive mutation that produces abnormalities in a variety of CNS structures including the hippocampus and the dentate gyrus (Wahlsten et al., '83, J. Hered. 74:421-425; Nowakowski and Wahlsten, '85, Anat. Rec. 211: 140A). The mutation sst is particularly interesting because it has differential penetrance (i.e., the sst/sst genotype does not always produce the same phenotype), and a variety of abnormalities have been observed. These abnormalities include complete or partial absence of the granule cell layer of the dentate gyrus, the presence of ectopic pyramidal and granule cells in the stratum oriens, variations in pyramidal cell number in area CA3, and reductions in the thickness and apparent cell density in the entorhinal cortex. The occurrence of these abnormalities varies not only from mouse to mouse but also from one side of the brain to the other. Thus, any one of the abnormalities listed above may be present on one side of the brain, whereas the other side of the brain may appear virtually normal. At present we do not have sufficient numbers of animals to determine if the abnormalities in different portions of the hippocampal region occur independently or if there are any right-left preferences.

Tritiated thymidine experiments show that in the case of the reductions in thickness and cell density in the entorhinal cortex the side with the reduced number of cells lacks the late-generated cells that would normally occupy the most superficial strata, whereas the side with the apparently normal number of cells in the entorhinal cortex has the late-generated cells. This observation suggests that the influence of the sst/sst genotype in the entorhinal cortex is on cell proliferation, although the role of differential cell death has not yet been explored.

Previously, we have reported that the abnormalities in the hippocampus and dentate gyrus are also the result of developmental deficits in both neuronal proliferation and neuronal migration (Nowakowski and Wahlsten, '85). Thus, taken together, these data suggest that the asymmetric development of the hippocampal region occurs relatively early in the developmental process. It seems likely that the asymmetry produced by the sst/sst genotype is not a normal feature of the hippocampal region, although it is also possible that it is acting by amplifying asymmetries that are normally present but masked by other features of the mature structure. In either case the availability of a mutation in which clear asymmetries are present may be a useful tool for analyzing the normal development of asymmetries in the CNS.

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292.8 THE WEAVER MUTATION: REGIONAL VARIATIONS IN CEREBELLAR DEVELOPMENT AND PROPOSED EARLY TIME OF GENE ACTION. K. Herrup and E. Trenkner. Dept. Human Genetics, Yale Med. School, New Haven and Dept. Pharmacology, NYU Medical Ctr., New York City.

Weaver (wv) is an autosomal mutation of mice. Homozygotes express a characteristic ataxia first noticeable in the second postnatal week. The most obvious structural corollary to the behavioral defect is the abnormal development of the cerebellum. The major pathological deficiencies reported to date are 1) an abnormal development of the Bergmann gial cell; 2) a defective postnatal migration of the granule cell neurons from their site of genesis in the external granular layer (EGL) to their final adult position in the internal granular layer (IGL); 3) the postnatal degeneration of most granule cells; and 4) the misalignment of the cerebellar Purkinje cells accompanied by a deficit in their numbers.

In our study of the weaver abnormalities, we have focussed on the developmental interaction of the Purkinje and granule cell, and have uncovered several previously undescribed anomalies. First, viewed from its dorsal aspect the patterns of cerebellar foliation appear haphazard (although bilaterally symmetric) with a distinct sulcus marking the midline. Second, although in midline sagittal sections the foliation resembles the wild type pattern, in sagittal sections from the hemispheres, the folia are chaotic in appearance due, in part, to a dorsal overgrowth of cerebellar tissue. This finger like extension of one hemispheric folium tends to run predominantly anterior-posterior with many medial extensions. The ectopic growth appears as if it compresses the more regular cerebellar tissue beneath it. Third, as late as postnatal day 35, the lateral cerebellum, particularly the flocculus/paraflocculus, has a modest population of granule cells in a semblance of an IGL. Fourth, the regional variation suggested by these observations is further emphasized by the density of Purkinje and Golgi II cells. Cell counts reveal that, while the number of cells per section is down by half at the midline, the value in the hemispheres is nearly normal. Overall we observe a 40% decrease in number.

We believe the data point to a mechanism of gene action that involves the Purkinje as well as the granule and Bergmann glial cell. The spectrum of defects suggests that the mutation interferes with the normal migration of both neuronal types beginning at early times in cerebellar development.

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292.9 STAGGERER MUTANT SHOWS DEMINISHED EXPRESSION OF TWO PURKINJE CELL-SPECIFIC PEPTIDES. J.R. Slemmon*, J.I. Morgan* and Dan Goldowitz. (SPON: R.E. Wimer) Div. Neurosci., Beckman Rsch. Inst., Duarte, CA, 91010; Phys. Chem. & Pharm., Roche Inst. Mol. Biol., Nutley, NJ, 07110 and Dept. Anat., Jefferson Med. Coll., Philadelphia, PA, 19107.

Two Purkinje cell-specific peptides (cerebellin and des-ser$^1$-cerebellin) were previously isolated and sequenced from rat and mouse (PNAS USA 1984,81; 6866-6870). Both chemical and immunocytochemical studies demonstrated that the expression of these peptides was developmentally regulated in the cerebellum. Chemical analyses of the cerebellins in Purkinje cell mutants (PCD, nervous) demonstrated only moderate losses of peptide expression, while in other cerebellar mutants (Weaver, Reeler), where primary gene action is believed to be extrinsic to Purkinje cells, there was a precipitous loss of peptide expression. Immunocytochemical localization of the cerebellins in Weaver and Reeler confirmed the chemical analyses. The Purkinje cells in these animals showed restricted peptide localization that was largely confined to regions with dense clusters of granule cells in Reeler and in the flocculo-nodular lobe in Weaver and Reeler.

These earlier studies suggested that cerebellin expression was at least partially dependent on granule cells and that the mechanism may involve synaptic contact. To examine this possibility, Staggerer (15, 30 and 90 days old) was employed since the genetic lesion in this animal results in an altered dendritic spine that interrupts the establishment of these synaptic contacts. Immunocytochemical localization in the control mice showed a typical expression profile for the cerebellins. In contrast, the Staggerer contained no cerebellin-positive Purkinje cells in any of the different aged animals examined. These results were unexpected in the 15-day-old mutant since a large number of granule cells were still present, and the majority of these had successfully migrated toward the internal granule cell region.

The results from this investigation suggests that synapse formation between granule cell and Purkinje cell neurons is therefore required for the normal expression of the cerebellins. The mechanism by which cerebellin levels are maintained does not appear to involve environmental conditioning, but rather a localized process that requires the establishment of synaptic contacts.

292.10 EARLY CORTICAL LESIONS ALTER CEREBRAL MORPHOGENESIS AND CONNECTIVITY IN THE RAT. B. Kolb and D. van der Kooy, Dept. of Psychology, Univ. Lethbridge, Lethbridge, AB., Canada, T1K 3M4.

Rats with neonatal removal of the frontal, motor, or parietal cortex are behaviorally different from rats with similar lesions in adulthood. Although they show spared functions on some learning tasks, the animals show severe impairments at most species-typical behaviors and complex spatial learning tasks. A possible reason for these data is that the early lesions produce changes in the development of the remaining brain. This study examined the gross morphology and cytoarchitectonics of the cortex, as well as some of its connections following neonatal cortical ablations. Rats were given bilateral lesions of the frontal or parietal cortex or complete hemidecortication at 1, 5, 10, or 100 days of age. The retrograde tracers true blue, propridium iodide, or nuclear yellow (.1 µl) were injected into the neocortex, striatum, or hippocampus of different animals in adulthood.

There were striking changes in both the morphology and connectivity in the neonatal operates, the effects being largest in the youngest operates. First, the earlier the lesion, the smaller the adult brain. Second, in the bilateral lesion groups, the earlier the lesion, the thinner the residual cortex. In contrast, for the hemidecorticates, the earlier the lesion the thicker the cortex, Third, acetylcholinesterase staining showed the normal patchy striatal organization was still present, even in the complete absence of cortical afferents in the hemidecorticate group. Fourth, details of the cortical cytoarchitecture were altered in both Nissl and myelin preparations. Fifth, early lesions altered the structure and connectivity of the thalamus, the earliest lesions having the greatest effect. Sixth, there were changes in thalamocortical organization, the most striking change being the presence of many labeled cells in the lateral geniculate nucleus following injections into the sensorimotor cortex of rats with frontal lesions. Seventh, there was an abnormal projection from the substantia nigra (compacta) to the sensorimotor cortex just posterior to the lesion boundary in the 1-day, but not older, frontal operates. Lucine injected into the nigra of 1-day frontal lesion rats also produced anomolous labeling in the parietal cortex. Finally, there were anomolous projections from the striatum ipsilateral to the early hemidecortication to the contralateral prefrontal cortex. In addition, the nigrostriatal projection was organized differently on the side ipsilateral to the lesion. These anatomical abnormalities may account both for why early cortical lesions may have different effects than adult lesions and why 1-day lesions have far more severe effects upon behavior than do later lesions.

292.11 LECTIN BINDING SITES IN THE POSTNATAL MOUSE CEREBRAL CORTEX: EPHEMERAL APPEARANCE OF THE BARREL FIELD. N.G.F. Cooper* and D.A. Steindler. (SPON: B.J. McLaughlin). Dept. Anat., Univ. Tenn. Ctr. Health Sciences, Memphis, TN 38163

Plant lectins bind to sugar residues of oligosaccharides, and enzyme linked lectins can therefore be used to histochemically localize cellular glycoproteins. We have shown that lectin affinity changes occur globally within the cerebrum and cerebellum during postnatal development (Cooper and Steindler, Neurosci. Abs., 10:43, 1984). The objectives of the present study were to define developmentally regulated changes in lectin affinities within a specific region of the cortex, namely, the primary somatosensory cortical barrel field. The somatotopic representation of the contralateral whisker pad in this region has been extensively studied, and it is recognized as a useful model for studying CNS pattern formation and plasticity. Parasagittal and flattened tangential vibratome sections from aldehyde-fixed cortex were treated with peroxidase-conjugated lectins. Following peroxidase histochemistry, the sections were processed for light and electron microscopy (LM and EM). At the LM level, we observed lectin binding in the barrel field of postnatal days 3 through 6 animals. The lectins bind with greater affinity to the presumptive sides rather than the centers of the individual barrels at a time when cells are evenly distributed throughout this region as judged by cresyl violet staining. The absence of a lectin delineated barrel field in postnatal days 1 and 2 suggests that this region develops its unique topography later than other areas of the cerebrum, described previously, and the cerebellum (Steindler and Cooper, Neurosci.Abs., 11, 1985) where discrete lectin bound laminae are present on day 1. While the barrel field is readily demonstrated with cresyl violet staining in the adult cortex, we were unable to demonstrate its presence with lectins in the adult. The ephemeral appearance of the barrel field suggests a critical period in development with respect to the synthesis of glycoconjugates recognized by these lectins. This period may be correlated with the development of a topographic pattern of innervation. Preliminary EM analysis demonstrates that lectin binding is predominantly related to the neuropil, being present on and between membrane surfaces of growing axons and dendrites. Supported by USPHS grants EY 02708 (N.G.F.C) and NS 20856 (D.A.S.).

292.12 LECTIN AND CHOLERA TOXIN BINDING SITES IN THE POSTNATAL MOUSE CEREBELLUM. D.A. Steindler and N.G.F. Cooper*. Div.Neurosci. Dept. Anat. Univ. Tenn. Ctr. Health Sciences, Memphis, TN 38163

Neuronal and glial cell surface glycoconjugates, and possibly extracellular glycosoaminoglycans, are presumed to play a role in the development of pattern formation in the CNS. We have used the binding properties of lectins and cholera toxin to determine if their binding to glycoproteins and gangliosides labels prospective cytoarchitectonic features in the early postnatal mouse cerebellum. Vibratome sections of postnatatal day (P) 1, 3 and 7, and adult mouse brains were incubated in peroxidase-labeled wheat germ agglutinin (WGA), peanut agglutinin (PNA), concanavalin A (Con-A), cholera toxin (CT), and radiolabeled WGA([N-acetyl-3H]). Control sections were incubated with lectins in the presence of various sugar haptens (e.g. N-acetylglucosamine, chitotriose, N-acetylgalactosamine, a-methyl mannoside, mannan) at different concentrations. Competition experiments were also performed by way of incubating sections with both peroxidase-labeled or unlabeled lectins (or CT) and WGA, N-[acetyl-3H]. Tissue sections were processed for histochemistry and/or autoradiography and examined under the light and electron microscopes.

The labeling patterns of the P1-P7 cerebellar cortex appear similar with all three lectins and CT, in contrast to that seen in the adult where different lectins and CT label different cellular layers. Most striking is the presence of an intense band of lectin and CT binding in the developing molecular layer. Electron microscopy reveals a pronounced labeling of young parallel fibers in this region. CT binding within the internal granule cell layer is patch-like beginning between P3-P7, and in the adult such labeling is similar to that seen in other studies using antibodies to gangliosides such as GD3 (Graus et al., Brain Res.324:190,'84). Negative staining of such patches with lectins such as Con-A indicates differences in ganglioside versus glycoprotein synthesis and distribution. Double labeling studies using peroxidase-labeled and radiolabeled lectins reveal the development of complementary labeling patterns within cortical layers as well as the deep cerebellar nuclei- e.g. in the adult, WGA and CT binding produces heavy labeling of the molecular layer; Con-A labels Purkinje and deep nuclear cells, whereas WGA binding in the deep nuclei is distinctively punctate and mostly perisomatic.

These results indicate that lectins and toxins recognize the development of distinct cytoarchitectural features in the cerebellum. The presence of variation in intensity between folial crowns and fissures of a lectin and CT positive band within the developing molecular layer, for example, adds credence to a notion of lectin and toxin recognition of glycoconjugates involved in neuronal migration, alignment, and synaptogenesis. Supported by USPHS grants NS-20856 (DAS) and EY 02708 (NGFC).

292.13 LECTINS AS MARKERS OF DEVELOPMENT IN THE RAT BRAIN T.J. DeGrauw* and B.H. Liwnicz. Division of Pediatric Neurology, Children's Hospital Medical Center and Department of Pathology, University of Cincinnati Medical Center, Cincinnati, Ohio 45229.

Biochemical studies have shown qualitative and quantitative changes of central nervous system (CNS) carbohydrate moieties during brain development (Margolis) et al, Brain Research, 112:363, 1976). Raedler and Raedler (Anat. Embryol, 162:21, 1981) have demonstrated an alteration in lectin affinity during the fetal development of rat brain. This study lacked later stages of brain development. We evaluated the binding of four lectins to rat brain at several fetal and postnatal stages of development. Rat brains at gestational days 13 (G13), 16(G16), 22(G22) and at newborn days 5 (N5) and 11(N11) were fixed in buffered formalin, slices were embedded in paraffin and 5 um sections were stained with concanavalin A (ConA), Ulex Europaeus Agglutinin I(UEAI), Wheat germ Agglutinin(WGA) and Peanut Lectin(PNL). Immuno-cytochemical staining was performed using biotinylated lectins in an Avidin-Biotin Complex(ABC) with and without neuraminidase digestion. At G13 the ventricular layer stained positively with all lectins. WGA stained bloodvessels and Con A bound diffusely to all brain structures. The ventricular layer at G16 showed the same staining pattern. In addition we found that UEAI bound to the majority of cells and cell processes and PNL stained scattered cells. Neuraminidase treatment did not alter the staining pattern. A clear change was seen at G22. PNL did no longer bind to the ventricular layer, positive staining was however evoked after digestion with neuraminidase. The number of cells, labeled by UEAI decreased progressively at G22 and N5. At this stage the pyramidal layer of the hippocampus did no longer stain. The pattern of WGA and Con A did not differ from earlier stages, but PNL stain started to diminish. However, pretreatment with neuraminidase increased PNL binding. The weakening of PNL staining continued at the next stage of development and at N11 a remarkable enhancement was seen after neuraminidase digestion. Other changes at this age included the absence of UEAI binding and a patchy distribution of Con A and WGA in cortex and other gray matter.

Lectins showed differential binding to rat brain at different stages of development. It appears that they can be useful in following structural changes of the developing CNS.

292.14 SYNAPSIN I IMMUNOHISTOCHEMISTRY DEMONSTRATES SYNAPTIC DEVELOPMENT IN THE RAT SUPRACHIASMATIC NUCLEUS. M.F. Bernstein and R.Y. Moore, Depts. of Neurology and Neurobiology, SUNY at Stony Brook, N.Y. 11794.

Synapsin I is a neuron-specific phospho-protein that is an endogenous substrate for cyclic AMP-specific and Ca/calmodulin-dependent protein kinases. Synapsin I is concentrated in nerve terminals in both the central and peripheral nervous system where it is associated with small synaptic vesicles (Navone et al. 1984).

The suprachiasmatic nucleus (SCN) is a late forming hypothalamic nucleus; perikaryal birth occurs between embryonic day 13 (E13) and E17 (Altman and Bayer 1978). Circadian rhythmicity in 2-deoxyglucose uptake appears in the SCN at E19, an age when ultrastructural analysis indicates that few synapses are present in the nucleus. The maternal circadian system entrains the fetal clock but the mechanism by which maternal influences are mediated is unknown (Reppert and Schwartz 1984).

In order to better understand the relations between the development of circadian rhythmicity and synaptogenesis in the SCN, synapsin I immunoreactivity was studied in coronal sections of the SCN from E19 through P10. Results from this study were compared with a previous electron microscopic (EM) study (Bernstein and Moore 1982) in which two counts were made from the SCN, total synaptic counts from micrographs scattered evenly throughout each coronal section and area counts from subdivisions of the nucleus. There are few immunoreactive elements in the E19-P2 SCN and this is corroborated by the EM total counts of synaptic terminals. At P4 synapses appear along the SCN-optic chiasm interface both in EM material and in the immunohistochemical material. The numbers of synapses increases until P8 when an essentially adult pattern is achieved. The virtual identity between the development of synapses in the EM material and the synapsin immunolabeled material indicates the power of the immunohistochemical method for the demonstration of synapse formation.

In other areas of the brain, for example, paraventricular nucleus, neostriatum, cortex and hippocampus, there is a distinctive pattern of development of synapsin immunoreactivity that differs from that in SCN. In those areas there is early staining of perikarya followed by development of densely stained terminal fields. These observations suggest that, as synaptogenesis is initiated, synapsin I is produced in perikarya and transported to axon terminals as synapsin is formed.

In conclusion, this study indicates that synapsin I immunohistochemistry provides an effective method for studying synaptogenesis.

We are grateful to Drs. Paul Greengard and Charles Ouimet for providing the synapsin I antibody. This study was supported by NIH grant NS-17600.

292.15 THE POSTNATAL DEVELOPMENT OF ANTIGENIC SAGITTAL BANDS IN THE CEREBELLAR CORTEX BY SELECTIVE REPRESSION OF IMMUNOREACTIVITY R. Hawkes* and N. Leclerc* (SPON: L. Poirier). Lab. of Neurobiology and Dept. Biochemistry, Laval University, Quebec, Canada G1K 7P4.

Monoclonal antibody mabQ113 recognizes a single polypeptide, apparent molecular weight 120 Kdaltons. In the rat cerebellum, immunoreactivity is confined exclusively to the Purkinje cells. Deposits of reaction product are found throughout the cytoplasm including the dendrites and dendritic spines, the cell body, the axons and the recurrent axon collaterals. No other cell types in the cerebellum are immunoreactive. Monoclonal antibody mabQ113 can distinguish between two classes of Purkinje cells. The mabQ113$^+$ cells are arranged in parasagittal bands which run throughout the cortex interdigitated by bands of mabQ113$^-$cells. Serial reconstructions reveal 15 bands of mabQ113$^+$ cells, one which straddles the midline and seven disposed symmetrically to either side.

We have now studied the postnatal development of mabQ113-bands. In the newborn rat (PO) there is no mabQ113-immunoreactivity. Staining first begins at around P7 and mabQ113$^+$ cells are first seen in the vermis. By P12 immunoreactivity is found throughout the cerebellar cortex. At P12 all the Purkinje cells are mabQ113$^+$. Parasagittal bands of staining emerge between P12 and P18 by the selective repression of immunoreactivity in the cells destined to become the mabQ113$^-$ bands. The adult display of bands is achieved at around P30.

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292.16 ABNORMAL CEREBELLAR FOLIATION FOLLOWING NEONATAL ADMINISTRATION OF METHYLAZOXYMETHANOL ACETATE: ANALYSIS OF PARALLEL FIBER ORIENTATION. D. E. Hillman and S. Chen. Dept. of Physiol. & Biophys., New York Univ. Med. Ctr., New York, NY 10016

A single 20 mg/kg injection of methylazoxymethanol acetate (MAM) within 24 hours of birth has been reported to produce ataxia and tremor in rats similar to the agranular cerebellum in genetic mutants or that induced by x-irradiation, virus or other chemical agents. However, our application of the procedure resulted in a large variability in the size of the cerebellum without noticeable behavioral abnormality except a slight tremor. Most unusual was a marked abnormality of the foliation persisting into adulthood regardless of the size of the cerebellum. The characteristic of this foliation was a cauliflower appearance formed by small blebs that could not be followed easily on the surface in any direction.

The cerebella were weighed and treated for Golgi impregnation and routine light microscopy. Serial-section reconstruction was used to determine the continuity in the lamination. We divided the cerebella into 3 groups as based on their weight: severe, moderate, and normal to mild. These size groups paralleled the reduction in number of granule cells. The most severely affected cerebella were reduced to half the size of controls and consisted of primitive lobules with a pebbly surface. Many folia lacked granule cells and consistently had a dispersion of Purkinje cells. The moderate group revealed a thinning of the molecular layer containing many ectopic granule cells. Many Purkinje cell somas were located in the internal granule layer. The mild group had a near normal lamination with only a few ectopic Purkinje cells. The folial pattern was the most complex in this group. The cortical layer was traced as a continuous sheet in serial sections.

The orientation of the parallel fibers seen in Golgi preparations and electron microscopy was variable in direction but primarily remained in parallel bundles following the prominent axis of the blebs. At the interception point between bundles, the parallel fibers crisscrossed or turned to merge with the larger bundle.

The mal-alignment of folia and parallel fibers was associated with perturbations in the proliferation of precursors in the external granular layer (EGL) and did not affect fiber direction once the parallel fibers began to form. This early effect on the EGL is a patchy survival of these precursors and is seen at postnatal days 5-7. A differential alignment of granule cell precursors in the patches may be responsible. Other mechanisms may involve the granule cells themselves or Bergmann glia. [Research supported by USPHS grant NS-13742 from NINCDS].

292.17 EARLY ABLATION OF THE SUPERIOR COLLICULUS DECREASES THE SIZE BUT NOT THE NUMBER OF LAYER V VISUAL CORTICAL CELLS. S. L. Pallas, K. C. Wikler and B. L. Finlay, Section of Neurobiology and Behavior and Department of Psychology, Cornell University, Ithaca, NY 14853.

Target availability is an important factor in the regulation of cell survival in the nervous system. For example, the amount of motoneuron survival in the vertebrate spinal cord parallels increases (Hollyday & Hamburger, J. Comp. Neurol., 1976) or decreases in target availability (Hamburger & Levi-Montalcini, J. Exp. Zool., 1949). The situation may be different for neurons lying completely within the central nervous system, however, because an individual neuron is both a target and a source of afference, and because central nervous system neurons often have collaterals projecting to more than one target. Mechanisms other than target control of cell survival are thus likely to operate in the central nervous system. In order to address this question, we have studied the response of pyramidal cells in layer V of visual cortex to removal of one of their principal targets, the ipsilateral superior colliculus, in the hamster. The hamster is particularly useful for this study because cortical cell death occurs postnatally.

Unilateral tectal lesions were made in neonate hamsters within 12 hours after birth. Lesioned animals were then killed on each of postnatal days 4-10, the major period of cell death in the cortex. The brains were fixed in formalin-alcohol, embedded in paraffin, and cut at 10μm. Serial sections were collected and stained with cresylecht violet. Lesions were 60-100% in extent, as verified by reconstruction. Sections through visual cortex (defined by reference to the adult) were examined and counts were made of degenerating cells, cells in layer V, and total cell number for both the lesioned and intact sides of the brain. We did not detect any differences in the normally low cell death rate between the lesioned and intact side (9.49 death index, lesion side vs. 11.16, intact side). Similarly, number of cells in layer V (748.7 cells/mm ± 164.0 (s.e.) lesion side vs. 675.4 cells/mm ± 133.5 intact side) and total cell number (3260 cells/mm ± 820.4 lesion side vs. 2840 cells/mm ± 466.8 intact) in the lesioned animals did not differ. However, we did observe a relative decrease in size of layer V pyramidal cells. The average diameter of pyramidal cells on the lesion side was 10% less than the diameter of cells on the intact side (14.6μm ± .22 lesion side vs. 16.3μm ± .25 intact side, n = 310).

Our results demonstrate that cells in lamina V of the visual cortex respond to loss of one of their principal targets not by an increase in cell death, but by a shrinkage in soma size. Death may be prevented by retention of collaterals projecting to other targets. This hypothesis will be tested with horseradish peroxidase injections into the pons following the period of synaptogenesis.

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292.18 EARLY THALAMIC LESIONS INCREASE NEONATAL CELL DEATH AND ALTER ADULT CYTOARCHITECTURE IN THE NEOCORTEX M. S. Windrem* and B. L. Finlay, Department of Psychology, Cornell University, Ithaca, NY 14853

Mammalian neocortex varies regionally both in number and in relative proportions of pyramidal and non-pyramidal cells in a unit cortical column. We have hypothesized that these regional differences in adult neocortical cytoarchitecture could be produced in part by differential cell death during development (Finlay and Slattery, Sci., 219, 1983). The factors that control neocortical cell death are thus of interest. In this study we assess the roles of afference and target availability on early cell death and adult cell number and type in hamster neocortex.

Electrolytic lesions were made in thalamic nuclei on the day of birth, prior to the establishment of thalamocortical connectivity and laminar differentiation in the neocortex. The cortical projection areas of the lesioned nuclei were assessed for cell death rates in neonates and for cell number and laminar distribution in a cortical column in adults. Cell numbers and cell death rates were assessed before (postnatal days 4 and 5) and at the peak (postnatal day 7) of the normal cell death period, and cell number and type were assessed at adulthood. In a second experiment to trace the fate of neurons destined for layer 4 after thalamic lesions, pregnant hamsters were injected with $^3$H thymidine on embryonic day 14 when the cells of cortical layers 2, 3 and 4 are generated.

Total cortical area is decreased ipsilateral to the lesion in both neonates and adults, and in the adult, total neuron number per unit cortical column is reduced in variable amounts (10 to 40%) depending on lesion size. The superficial layers, 2-4 combined, were reduced by one third; particularly, layer 4, the principal thalamic input area, was barely discernible. In an adult with a small lesion, layer 6 was normal; while in an adult with a large lesion, the number of cells in layer 6 was reduced by half. The number of cells in a column remained constant only in layer 5, which is neither afferent nor target for the thalamus.

The amount of cell death in the neocortical areas investigated here is normally quite low. Cell death increase was seen at all postnatal days, including the earliest postnatal day in which normal cell death has not yet begun (P4 and P5, when normal rates are close to 0, 12.5 times higher on the ipsilateral side than the contralateral side; P7, 3.3 times higher on the ipsilateral side). In the autoradiography experiment, by P7, the numbers of labeled live cells were still unchanged, though the numbers of labeled dead cells more than doubled in the ipsilateral cortex.

These results indicate that early loss of thalamic input and target availability profoundly alter adult neocortical cytoarchitecture. Part of this alteration must occur through differential cell death. Whether the apparent disappearance of layer 4 is entirely due to increased cell death, or also to changed cell morphology is presently under investigation.

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293.1 MPTP TREATMENT DECREASES NIGRAL LEVELS OF REDUCED GLUTATHIONE. T.N. Ferraro, G.T. Golden, R.G. Fariello, and T.A. Hare. Depts. Pharmacology and Neurology, Thomas Jefferson Univ., Phila., PA 19107 and Neurology Service, VA Medical Center, Coatesville, PA 19320.

Administration of the synthetic compound 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) has been documented to produce a parkinsonian syndrome in both humans and animal models. To elucidate the mechanism underlying the selective toxic effect of MPTP on substantia nigra (SN) neurons, an ultrasensitive HPLC method was used to study over 30 amino acids and related compounds (AA) in SN and caudate/putamen (C/P) from control and MPTP-treated Long-Evans rats. Animals were given one daily injection of MPTP (25 mg/kg ip) for 15 days, and sacrificed 24 hr after the last dose. Brains were removed in < 1 min, dissected on ice and discrete anatomical regions frozen immediately; SN and C/P death-to-freezing intervals were < 3 min. Tissues were homogenized in 0.4M PCA, centrifuged and supernatant fluids filtered prior to AA analysis. Baseline studies in untreated rats documented characteristic AA profiles in SN and C/P. MPTP-treated rats exhibited significantly lower levels of Asp and reduced glutathione (GSH) in SN and significantly higher levels of Tyr, Phe, Trp, Lys and ethanolamine in C/P compared to controls. An interaction of MPTP with GSH may be related to its selective action on SN neurons since it has been documented that for toxicity to develop, MPTP must be enzymatically converted to $MPP^+$, a reactive electrophile suspected of setting up a powerful oxidation center in the brain. Compared to other brain regions, normal SN is characterized by low levels of GSH, rendering this region particularly vulnerable to insult.

293.2 ARGYROPHILIC DEGENERATION OF SUBSTANTIA NIGRA NEURONS IN C57 MICE DUE TO 1-METHYL-4-PHENYL-1,2,5,6 TETRAHYDROPYRIDINE (MPTP). R.C. Switzer, III. R.H. Cole Neuroscience Lab., Depts. Pathology and Medical Biology, University of Tennessee Memorial Research Center and Hospital, Knoxville, TN 37920

Intake of MPTP by humans causes Parkinson's disease (PD)-like symptoms which are treatable with standard L-dopa therapy. Primates and some rodents given MPTP display cerebral biochemical and pathological changes, including loss of the dopaminergic (DA) neurons of the pars compacta of the substantia nigra (pcSN), similar to PD, thereby suggesting that MPTP may be useful as an animal model for PD. However, adequate neuroanatomical evidence is lacking to show that the affected DA neurons in pcSN are the only DA neurons affected.

To determine what areas of the brain show neuronal cell death after treatment with MPTP, brain sections from 9-month-old male C57 mice were stained with the degeneration-sensitive cupric-silver stain of de Olmos. The mice were given MPTP-hydrochloride i.p. 30 mg/kg on a schedule similar to that described in an earlier biochemical study (*Science* 224:1451, 1984). The mice were grouped to receive 1,2,5, or 10 daily injections and allowed to survive either 3 or 10 days after the last injection. Groups of 6-12 brains from treated mice were embedded in the same gelatin blocks, along with one untreated control brain, for freeze-sectioning. In cupric-silver stained sections, degeneration, represented by characteristic argyrophilic neuron cell bodies, was found in the pcSN but not in the reticular part of SN. The degeneration of neuron cell bodies was most prominent in mice that survived 3 days after receiving either 1 or 2 injections. In striatum, the argyrophilic grain pattern typical of degenerating axon terminals, apparently representing the degenerating terminals of the nigra-striatal projection, was also best seen in the 3 day survival group. In those mice surviving 10 days, little or no argyrophilic debris was found, as was also true of all mice receiving 5 or 10 doses. No degenerating neurons were observed in the median eminence where other dopaminergic neurons reside.

These findings indicate three important points: 1) in MPTP-treated C57 mice, the only neurons destroyed are those of the pcSN, thereby providing some degree of validation of the MPTP-treated mouse as an animal model of PD; 2) the neuronal loss due to treatment with MPTP can occur early with only one injection, as indicated by the appearance of argyrophilic neuron cell bodies, a finding consistent with decreased dopamine in the striatum (ibid); and 3) the cupric-silver staining method is useful in the early detection of neurotoxic effects that are lethal to neurons, as well as providing a means to efficiently survey the brain for affected sites. This study was supported by the Robert H. Cole Neuroscience Foundation.

293.3 ASCORBIC ACID ATTENUATES THE NEUROTOXIC EFFECTS OF METHAMPHETAMINE, 6-HYDROXYDOPAMINE (6HDA) AND 1-METHYL-4-PHENYL-1,2,5,6-TETRAHYDROPYRIDINE (MPTP). R.M. Carelli*, M.F. Jarvis and G.C. Wagner. Dept. of Psychology, Rutgers University, New Brunswick, NJ 08903

Methamphetamine, 6-HDA and MPTP are potent central nervous system toxins causing; 1) depletion of monoaminergic neurotransmitters; 2) a decrease in dopamine uptake pumps; 3) a decrease in tyrosine hydroxylase activity; and 4) neuronal degeneration. It has been speculated that the mechanism through which each of these agents destroys neurons is the generation of hydroxyl radicals which, in turn, cause lipid peroxidation leading to membrane disruption and cell death. The formation of hydroxyl radicals can be limited by antioxidants such as ascorbic acid. Accordingly, the following study was conducted to determine if treatment with ascorbic acid attenuates the toxic actions of these compounds.

Subjects were 72 adult male Long-Evans rats housed individually with free access to food and water. They were randomly divided into nine groups of eight rats each. All rats received unilateral lesions of methamphetamine (40 ug), 6-HDA (20 ug) or 1-methyl-4-phenyl pyridine ion $MPP^+$ (20 ug). The toxin was stereotaxically delivered in a 5 ul saline vehicle to the caudate nucleus under pentobarbital anesthesia. The unlesioned side served as control. Three of the groups received ascorbic acid (100 mg/kg) IP 20 min before and then 20, 60 and 120 min after delivery of the toxin. Three of the groups received ascorbic acid (1000 mg/kg) in an otherwise similar regimen. Finally, the last three groups received only the toxin. All rats were allowed to survive one week and were then sacrificed, brains removed and caudate nucleus dissected. Brain parts were stored in liquid nitrogen until assayed by HPLC with electrochemical detection.

Methamphetamine depleted dopamine (DA) to 63% of control. Ascorbic acid (100 or 1000 mg/kg) attenuated the methamphetamine-induced DA depletion (to 84% and 90% of control, respectively). 6-HDA depleted caudate DA to 30% of control. Ascorbic acid (100 or 1000 mg/kg) attenuated the 6-HDA-induced DA depletion (to 49% and 51% of control, respectively). Finally, $MPP^+$ depleted DA to 12% of control. Ascorbic acid (100 or 1000 mg/kg) attenuated the $MPP^+$-induced DA depletion (to 34% and 57% of control, respectively). Serotonin levels were not altered by any of the treatments.

These observations indicate that ascorbic acid loading reduces the neurotoxic actions of methamphetamine, 6-HDA and $MPP^+$. Besides other neurotoxins, the generation of hydroxyl radicals is thought to be responsible for the loss of neurons associated with aging. It is conceivable that ascorbic acid could be effective in these situations as well. However, based upon the facts that ascorbic acid did not completely protect the rats, and that only one antioxidant was used, caution must be exercised in the interpretation of these results.

293.4 EVALUATION OF THE RODENT MODEL OF PARKINSON'S DISEASE USING MPTP M.F. Jarvis, J.G. Rubin* and G.C. Wagner. Dept of Psychology Rutgers University, New Brunswick, NJ 08903

The systemic administration of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) causes destruction of nigro-striatal neurons. The toxic effects of MPTP are dependent upon its oxidation to the 1-methyl-4-phenyl pyridine ion ($MPP^+$) by MAO B. In humans and rhesus monkeys this neurotoxicity is accompanied by a behavioral symptomology similar to Parkinson's disease including resting tremor, rigidity, and akinesia. These behavioral symptoms as well as the depletion of striatal dopamine caused by MPTP are reversed by l-dopa administration. In rodents, neurochemical and histological evidence indicates that MPTP also causes nigro-striatal toxicity. However, MPTP is less potent in producing this effect in the rodent and, furthermore, the behavioral symptoms following MPTP are less severe in rodents. These latter observations have led some to question the utility of the MPTP/rodent model of Parkinson's disease. The following studies were conducted in order to evaluate the effects of $MPP^+$ on the shuttle box performance of mice. The shuttle box was chosen because, traditionally, this procedure has proven to be a sensitive screen to both dopaminergic toxicity and for neuroleptic actions.

Adult, male Rockland-Swiss mice served as subjects. Shuttle box training consisted of the following: mice were trained to respond (i.e. cross to the opposite side of the chamber) during a 20 sec buzzer (70 dB, 2500 Hz); in the absence of an avoidance response a scrambled 1.0 mA footshock was delivered until the animal either made an escape response or until 20 sec had elapsed (response failure); a 20 sec intertrial interval seperated each trial and mice received 20 trials/day.

All mice were trained to an 90% correct avoidance responding criterion/day for three days. Under these baseline conditions mice made an average of 19.6 $\pm$ 0.2 avoidance responses, 0.4 $\pm$ 0.2 escape responses, no response failures, and 19.2 $\pm$ 3.1 spontaneous crossings (during the ITI). Mice then received bilateral intra-caudate injections of 10 ug of $MPP^+$ delivered stereotaxically in a 3 ul saline vehicle. Following $MPP^+$ treatment, performance was significantly disrupted. Mice made 3.6 $\pm$ 1.4 avoidance responses, 7.2 $\pm$ 1.8 escape responses, 9.1 $\pm$ 2.6 response failures, and 2.3 $\pm$0.7 spontaneous crossings. L-dopa (400 mg/kg) reinstated shuttle box performance (9.2 $\pm$ 2.7 avoidance responses, 9.9 $\pm$ 2.5 escape responses, 0.8 $\pm$ 0.6 response failures, and 17.8 $\pm$ 8.5 spontaneous crossings). Finally, four seperate groups of mice received bilateral intra-caudate $MPP^+$ (0.0 or 10 ug/side). $MPP^+$ caused significant dopamine depletions (to 30% of control) and l-dopa (400 mg/kg, 30 min pretreatment) reversed this depletion to 127% of control. These observations substantiate the use of the rodent-MPTP model of Parkinson's disease.

293.5 EFFECT OF 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE (1-MPTP) ON NIGRAL SUBSTANCE P-LIKE IMMUNOREACTIVITY (SPLI).
L.A. Matsuda*, P.K. Sonsalla*, J.W. Gibb and G.R. Hanson. Dept. Biochem. Pharmacol. & Toxicol., Univ. of Utah, Salt Lake City, UT 84112.

1-MPTP has been reported to have neurotoxic actions on the catecholaminergic transmitter systems of several different animal species. Most notably, 1-MPTP selectively decreases dopamine concentrations and tyrosine hydroxylase (TH) activity in the same neuronal systems that are destroyed in patients suffering from Parkinson's disease and its use in laboratory animals has been proposed as a model for this neurological disorder. In addition to the significantly reduced dopamine concentrations found in the basal ganglia of parkinsonians, the concentrations of the neuropeptide, substance P (SP) in the globus pallidus and the substantia nigra also are significantly diminished (Mauborgne et al., Brain Res., 268:167, 1983). This decrease is thought to be a compensatory reaction to the loss of nigrostriatal dopamine neurons in these patients and likely reflects increased release of SP. In order to assess the validity of the MPTP animal model for parkinsonism, we examined the effect of this neurotoxin on the nigral concentrations of SP in both rats and mice.

In male C57Bl mice, 1-MPTP treatment (15 mg/kg, q 6 h; 4 doses) reduced nigral SP concentrations to 66% of control three days after treatment. This decrease appeared to be only transient, however, since at seven days after treatment the decrease of SPLI was not significantly different from control. Similarly, in male Sprague-Dawley rats whose striatal dopamine concentrations were decreased to 51% of control by constant infusion of 1-MPTP (42 mg/24 hr; 7 days after treatment), nigral SPLI was 76% of control ($p<0.05$).

We previously reported that coadministration of the dopamine receptor antagonist, haloperidol (HALO) enhances the effect of 1-MPTP-induced reductions of striatal dopamine concentrations and TH activity (Matsuda et al., Soc. Neurosci., 10:882, 1984). However, in both mice and rats the concurrent administration of HALO (2 mg/kg, q 6 h; 4 doses) with 1-MPTP prevented the 1-MPTP-induced decrease of nigral SPLI concentrations.

These data suggest that 1-MPTP treatment modifies the transmission of the striatonigral SP system in a manner similar to that seen in patients with idiopathic Parkinson's disease. However, it is unclear why the MPTP-induced responses of the DA and SP systems are contrarily affected by the concurrent administration of HALO.

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293.6 CLONIDINE PROTECTION FROM THE TOXICITY OF PERIPHERALLY AND CENTRALLY ACTIVE ACETYLCHOLINESTERASE INHIBITORS. R.S Aronstam and J.J. Buccafusco. Department of Pharmacology & Toxicology, Medical College of Georgia, Augusta, GA 30912 and Veterans Administration Medical Center, Augusta, GA 30910.

Echothiophate and soman are potent cholinesterase inhibitors whose toxic effects involve several systems. Soman affects both central and peripheral nervous systems, although it is not clear whether the primary site for acute lethality is central or peripheral. Echothiophate, on the other hand, does not cross the blood-brain barrier. The purpose of this study was to compare the symptoms of echothiophate and soman poisoning and to examine the protective effect of clonidine, a centrally-active alpha$_2$-adrenergic agonist, against each of these agents.

Echothiophate and soman were dissolved in saline and injected by a subcutaneous route into outbred, ICR mice (25-34 g) in a volume of 5 ul/g body weight. Lethal dose (LD)-response curves were constructed using 5 doses of inhibitor (8-12 animals/point). LD50 values were obtained by linear regression analysis of log dose vs probit of % lethality plots (the correlation coefficient was greater than 0.9 in all cases). Behavioral measures taken were: time to onset of whole body tremors (OTT), time to onset of loss of righting reflex (LRR), and the occurrence of Straub tail (ST), salivation (SAL), and muscle fasiculations (MF).

The LD50s for soman and echothiophate were 156 and 141 ug/kg, respectively. Tremor (OTT=3.52 min at LD90), ST (92% at LD90) and SAL (83% at LD90) were observed in soman-treated mice, but MF were never seen. Although acute respiratory paralysis was evident with both agents, no tremors were observed in echothiophate-treated mice and the occurrence of ST was low (6.3% at LD90). MF and SAL, however, occurred in all mice receiving an LD50 dose of echothiophate. Therefore, as expected, symptoms of peripheral toxicity (MF and SAL) predominated in echothiophate toxicity. With soman, central symptoms (ST and tremor) predominated and SAL was the only consistent peripheral symptom.

Pretreatment (20 min) with clonidine (300 ug/kg) reduced the toxic manifestations of an LD90 dose of soman. Lethality was reduced to 44%, OTT and LRR were increased 4 fold, and the occurrence of ST and SAL were reduced to 56 and 44%, respectively. In contrast, clonidine did not alter lethality or the occurrence of MF, ST and SAL caused by an LD90 dose of echothiophate. LRR, however, was increased (from 12.9 to 18.4 min).

These findings indicate a predominantly central site of action for the acute toxicity of soman in mice, and confirm our earlier finding that clonidine affords a selective protection against the central and peripheral muscarinic, but not peripheral nicotinic, toxic effects of cholinesterase inhibitors.

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293.7 ORGANOPHOSPHATES INCREASE DIHYDROPYRIDINE RECEPTOR SITES IN RAT BRAIN. N. Monis*, J. Valdes and D.H. Ross. (SPON. J.C. Flynn) Div. Molecular Pharmacology, Department of Pharmacology, University of Texas Health Science Center, San Antonio, TX 78284.

Voltage-dependent ion channels have been studied as sites of action for a wide variety of neurotoxins. Some of these neurotoxins specifically alter $Ca^{++}$ fluxes across neuronal membranes, which contribute to their pharmacological effects. One such toxin, maitotoxin, derived from a dinoflagellate (Gambierdiscus toxicus) is believed to activate $Ca^{++}$ channels leading to enhanced release of neurotransmitters (Takahashi et al, JBC 258:20944-10949, 1983). Recently, the dihydropyridine $Ca^{++}$ agonist BAY K 8644 has been reported to produce profound behavioral responses when administered in vivo (Bolger et al, Arch. Pharmacol. 328:373-377, 1985). These include limb clonus, tonus, arched back, Straub tail and increased sensitivity to auditory stimulation. Since $Ca^{++}$ content in nerve terminals is believed to increase during electroshock-induced convulsions, we suspected that organophosphates may alter $Ca^{++}$ movement in conjunction with their behavioral effects. We have monitored dihydropyridine receptor sites in rat brain following in vitro and in vivo administration. $Ca^{++}$ channel receptor sites were measured as nifedipine (1 μM), displaceable [$^3$H]nitrendipine binding in cortex synaptic membranes (1 hr) following a single injection of Soman or Sarin (50 μg/kg) and 24 hrs after a single injection. Control dihydropyridine receptor binding of 112 fmol/mg protein was increased (37.5%) to 154 fmol/mg protein 1 hr after Soman administration. 24 Hrs later, treated rats still exhibited an increased dihydropyridine receptor binding activity at 133 fmol/mg protein. Membranes from Soman- or Sarin-treated rats were treated with EGTA/EDTA to remove $Ca^{++}$ and $Mg^{++}$ ions and dihydropyridine binding was repeated. In stripped membranes without $Ca^{++}$ or $Mg^{++}$, dihydropyridine receptor binding was still increased following in vivo Soman (15%), while 1.5 mM $Ca^{++}$ and $Mg^{++}$ addition further increased receptor binding to 40% over control levels. In vitro dichlorvos (50 μM) or Sarin (10 μM) increased dihydropyridine receptor binding 40% and 25% over control. These results demonstrate that organophosphates increase $Ca^{++}$ antagonist receptor sites in rat brain. These findings suggest that organophosphates may produce some of their pharmacological effects by interaction with the dihydropyridine binding site influencing $Ca^{++}$ transport.

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293.8 BRIEF MALATHION EXPOSURE DURING THE LATE BRAIN GROWTH SPURT AND THE LONG-TERM EFFECTS ON MUSCARINIC AND HISTAMINERGIC RECEPTOR BINDING IN RAT BRAIN. K.E. Light, Colleges of Pharmacy and Medicine, University of Arkansas for Medical Sciences, Little Rock, Arkansas 72205

Exposure to organophosphate insecticides in the environment is becoming an increasingly common experience. Although much of the acute toxicity of these compounds is known, the chronic toxicity and particularly the effects of these compounds on developing central nervous system (CNS) receptor dynamics has infrequently been the subject of investigation.

The post-natal period of the rat presents an opportunity to investigate the effects of controlled drug exposure on the potentially vulnerable developing structures of the CNS. We decided, therefore, to expand our recent investigations concerning the effects of drug exposure during development to include malathion. CD-derived rat pups were exposed to malathion (50 mg/kg/day and 150 mg/kg/day) by sub-cutaneous injection (PN4-PN8) in a corn oil vehicle. Control animals received vehicle injections only. The pups were kept with lactating dams throughout the total postnatal period and were sacrificed on PN20. The brains were removed and dissected into the following regions: cortex, striatum, medulla-pons, cerebellum and diencephalon. $H_1$-histamine receptor binding was measured in the cerebellum by saturation analysis using ($^3$H)-pyrilamine with excess triprolidine to determine non-specific binding. The results show a dose dependent decrease (51%) in receptor site density with no significant alteration of receptor affinity.

In the striatum and cortex, muscarinic receptor binding was determined by saturation analysis using ($^3$H)-quinuclidinyl benzilate with excess atropine to determine non-specific binding. In both sections, malathion exposure did not produce significant long-term alterations of receptor binding. We conclude that malathion exposure during brain development can produce significant long-lasting alterations of selected receptor systems dependent upon the regional stage of brain development and the duration of exposure. (Supported by the UAMS Foundation Fund and NSF Grant ISP 801147)

293.9 PRODUCTION OF CONVULSIONS AND BRAIN DAMAGE BY MICROINJECTIONS OF ORGANOPHOSPHATES INTO RAT BRAIN. J.H. McDonough, M.T. Nipwoda*, M. Smith*, and C.G. McLeod*. U.S. Army Medical Research Institute of Chemical Defense, APG-EA, MD 21010-5425.

Systemic administration of certain organophosphates can produce convulsions and a disseminated pattern of brain damage. The present studies investigated the effects of direct microinjections of soman (GD) or VX, into discrete areas of the rat brain to possibly localize structures which may mediate these effects. Direct injections were performed using a stereotaxically guided microsyringe (MS) while rats were maintained under halothane/oxygen anesthesia or in conscious animals using chronically implanted cannula (CA).

Bilateral MS injections up to 11 nmol GD into the basolateral amygdala (BA) failed to evoke either proconvulsive or convulsive reactions (N=6). If rats were pretreated with lithium (LiCl 3.0 meq/kg/da, sc), similar injections of 11 nmol GD into BA evoked repetitive clonic convulsions and brain damage (N=3), varying degrees of proconvulsive behaviors (wet dog shakes, repetitive mouth movements, facial clonus) with no brain damage (N=6), or no effect (N=2). When a cholinergic agonist, carbachol (5.0 nmol), was coadministered with a dose of GD (5.5 nmol) which was ineffective by itself, both convulsive behavior and brain damage were evoked with either bilateral or unilateral MS injections into BA in animals which received no lithium pretreatments. Similar combined MS injections of GD and carbachol into the medial septum, dorsal hippocampus or nucleus basalis of Meynart did not evoke convulsive behavior nor produce brain damage.

Unilateral injections of VX (3.37 nmol) into BA using either the MS or CA technique were highly effective in eliciting convulsive reactions and brain damage. MS injections in four animals resulted in two developing convulsions, one demonstrating proconvulsive behaviors and one showing no reaction. The same dose of VX resulted in strong convulsions in five of seven CA subjects and proconvulsive behaviors in one other. All animals which convulsed had extensive bilateral pyriform cortex damage.

Brain damage was only observed in animals which developed sustained limbic convulsions after central injections of these compounds. The degree and extent of the damage in a given animal was positively related to the severity of the convulsion. These compounds do not appear to be directly neurotoxic since damage was not evident in animals which did not develop convulsions even though they received amounts of drug equivalent to those animals which did convulse and had damage. In addition, in the convulsing animals, areas in close proximity to the injection site (caudate, putamen, globus pallidus) were consistently spared damage while there was extensive destruction to distal structures (pyriform cortex, hippocampus) synaptically connected to the target area. The BA, a cholinoceptive area, is probably a critical structure for either initiating or sustaining limbic convulsions evoked by these anticholinesterase compounds.

293.10 TARGET SIZE OF NEUROTOXIC ESTERASE AS DETERMINED BY RADIATION INACTIVATION. C. D. Carrington*, D. J. Fluke*, and M.B. Abou-Donia. Duke University, Durham, North Carolina 27710.

The target size of neurotoxic esterase (NTE), the putative target site for the initiation of organophosphorus compound-induced delayed neurotoxicity, was examined by determining the rate at which the esterase activity was destroyed by irradiation. Samples of hen brain were prepared by slowly drying a microsomal preparation under vacuum. The dried samples were then irradiated with electrons from a 1 MeV Van Der Graff generator. The doses ranged from 0 to 24 Mrads. The radiation doses were calibrated by the rate of inactivation of T1 phage activity (MW=560K). Following the irradiation procedure, the samples were resuspended in buffer and enzymatic activity was measured. In eight experiments with NTE, the target size was determined to be 148±13 kilodaltons, or 138K if all the data were pooled. In three experiments with acetylcholinesterase (AChE), the target size was found to be 56±13K or 58K if all the data were pooled. There is evidence that the molecular weight of the catalytic subunit of AChE is about 70K. If AChE inactivation is used to calibrate the radiation dose, the target size of NTE is calculated to be 167K, which is very close to the apparent molecular weight (155-160K), as determined by SDS-gel electrophoresis, of the major diisopropylphosphoroflouridate binding protein with a sensitivity to organophosphorous inhibitors similar to that of NTE. The target size of NTE was found to be similar in two experiments with rat brain and one with cat brain. This study was supported in part by NIEHS grant ES0-2717 and NIOSH grant OH0 2003.

293.11 FINE STRUCTURE OF RAT HIPPOCAMPAL SLICES AND SOMAN INDUCED ACTIVITY F.J. Lebeda, K.C. Sikora-VanMeter, R.C. Wierwille and W.G. VanMeter. Dept. Neurology, Baylor Coll. Med., Houston, TX; Dept. Pharmacol. Exptl. Therap., School Med., Univ. Maryland, Baltimore, MD 21201; USAMRICD, APG, MD 21010; Dept. Vet. Physiol. and Pharmacol., Iowa State Univ., Ames, IA 50011.

Fine structure of rat hippocampal slice preparations were examined in male and female 100 gram Sprague-Dawley rats after SOMAN induced epileptiform activity. The rats were decapitated, the brains quickly removed, placed in chilled saline, and hippocampal slices prepared with a tissue chopper. The slices were transfered to a modified Haas chamber, and continuously superfused in oxygenated saline at 33 to 35 degrees C. Two sets of slices were prepared: (a) slices used for electrophysiological studies followed by fixation as described below and (b) slices used for morphology only. Control recordings by conventional methods were made from CA1 prior to 100 nM SOMAN exposure. Following SOMAN exposure of 10,30,60,120, or 180 min, epileptiform events of ca. 0.2 Hz in CA3 occur within 10 minutes of bath application. However, this activity decreases markedly with duration of exposure. For comparative analysis of fine structure, control slices were incubated in saline up to 5h followed by fixation in cacodylate buffered 5% glutaraldehyde. All slices were postfixed in OsO4, dehydrated, embedded in a Polybed812/Araldite mixture, and thin sections made from CA3. After 5h of incubation in control saline the slices show 2 layers: (i) an outer layer which mainly contains deteriorating cells and profiles and (ii) an inner layer with the majority of the neurons and neuropil intact in the presence of degenerated neurons and glial cells. Pyramidal neurons of all SOMAN exposed slices either show indentation or invagination of their nuclei, often coupled with the appearance of multiple nucleoli. Large lipid inclusions are seen within the soma of the CA3 neurons in slices preexposed to SOMAN for 5h before washout of the agents and reexposure. Furthermore, lamellar bodies, an early sign of degeneration, are present in the neuronal as well as glial elements of these slices. More degenerated neurons and glia are present in SOMAN exposed than in control slices. Moreover, the degree and frequency of degenerating profiles is also dependent upon the duration of the total exposure time. (See also NS. Abst., Sikora-VanMeter et al, this meeting)

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293.12 CHANGES IN COMPOSITION OF PROTEINS OF FAST AXONAL TRANSPORT IN RATS WITH ACRYLAMIDE NEUROPATHY. M.A. Bisby and J.D. Redshaw*. Department of Medical Physiology, University of Calgary, Calgary, Alberta, Canada, T2N 4N1.

Changes in the ultrastructure of spinal ganglion neurons following acrylamide administration superficially resemble those induced by axotomy (Jones, H.B. and Cavanagh, J.B., Neuropath. Appl. Neurobiol. 10: 101, 1984). Since axotomy of these neurons produces characteristic changes in the composition of fast-transported proteins (Redshaw and Bisby, Exp. Neurol. (in press), 1985) we examined the composition of fast-transported proteins in sensory and motor axons of the rat sciatic nerve following acrylamide administration.

Acrylamide (Fisher) was injected i.p. daily (50 mg/kg in 1 ml sterile saline/kg), with an equal concentration of bis-acrylamide used as a control. The development of neuropathy was assessed using the extent of hind limb splaying on landing from a 30 cm drop (Edwards, P.M. and Parker, V.H., Toxicol. Appl. Pharmacol. 40: 589, 1977). At various time intervals after beginning acrylamide treatment, fast-transported proteins were labelled by injecting L[$^{35}$S] methionine into either lumbosacral cord or dorsal root ganglia of the anesthetized rats. Transported proteins were collected proximal to a sciatic nerve crush and characterized by 1- and 2-dimensional PAGE, followed by fluorography. In addition, other rats received a distal sciatic nerve crush 7 days before L[$^{35}$S] methionine injection so that changes following acrylamide intoxication could be compared with those following axotomy.

As shown by behavioural testing, the neuropathy began around day 4 of acrylamide treatment and increased until day 10. In 10-day animals, changes were observed in the composition of transported protein in both motor and sensory axons. Our analysis of these changes concentrated on the 20-30 K dalton range where we had previously observed the most significant and reproductive changes following axotomy. We found that changes after acrylamide were both quantitatively less and qualitatively different from those following axotomy. In sensory axons no significant changes in composition were observed at 2, 4 or 7 days of acrylamide treatment, showing that the detectable changes in fast-transported proteins do not precede the development of the behavioural features of the neuropathy.

We suggest that the changes in composition of transported protein are a consequence of the neuropathy, rather than a cause. The earliest effect of acrylamide administration is a reduction in retrograde transport of NGF (Miller, M.S. et al., Toxicol. Appl. Pharmacol. 69: 96, 1983), so we propose that the changes we observed are part of the cell body reaction to loss of target-derived trophic factors.

293.13 GAPDH, PFK AND ATP IN MOUSE SCIATIC NERVE AFTER SINGLE DOSES OF ACRYLAMIDE SUFFICIENT TO SLOW RETROGRADE AXONAL TRANSPORT. Stephen M. Ross and Peter S. Spencer, Institute of Neurotoxicology, Departments of Neuroscience and Pathology, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

Impairment of retrograde axonal transport following single doses of acrylamide comprises the earliest detectable change in nerve-fiber integrity and precedes, by several days, the well-documented morphological alterations associated with repeated exposure to this neurotoxin. This study attempts to establish whether comparable single doses of acrylamide disrupt energy systems supporting retrograde transport in peripheral nerve. Single doses (100-300 mg/kg) of acrylamide or N,N'-methylene-bisacrylamide, a reportedly non-neurotoxic analog of acrylamide, failed to produce dose- and time-dependent alterations in the glycolytic enzymes, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), phosphofructokinase (PFK) and of the ATP levels in whole and desheathed mouse sciatic nerve. Only at the highest dose (300 mg/kg, 5 h, 30% mortality) were deficits in the enzyme activity of GAPDH and PFK recorded in desheathed nerve from acrylamide- and bisacrylamide-treated mice. At this dose and time interval, ATP concentration in whole nerve was non-significantly decreased by 22% and 32% by acrylamide and bisacrylamide, respectively, with no evidence of inhibition of GAPDH and PFK in whole-nerve post-nuclear supernatants.

These observations indicate that single doses of acrylamide, sufficient to inhibit retrograde axonal transport (100 mg/kg), fail to lower whole and desheathed nerve GAPDH, PFK and ATP levels. The interpretation of these results is hampered by the unknown contribution of axon and Schwann cell compartments to the values obtained for nerve. However, if axons contribute a small component of the total pool, then a significant change in axonal glycolytic function may be readily missed. If, on the other hand, axons make a major contribution to the total values, these data suggest that disruption of glycolysis does not appear to underly deficits of retrograde axonal transport produced by single doses of this neurotoxin.

293.14 ACRYLAMIDE ALTERS THE FUNCTION OF THE PRIMARY AFFERENT TERMINAL. Teresa DeRojas* and Barry Goldstein. Dept. of Pharmacology and Toxicology, Medical College of Georgia, Augusta, GA 30912.

Acrylamide (ACR) is a neurotoxic chemical affecting both the peripheral and central nervous systems. Previous studies have shown that the sensory nerves are more susceptible to the neurotoxic actions of ACR than motor nerves. Studies in this laboratory on the monosynaptic reflex suggest that ACR exerts its effect on the primary afferent terminal. These studies were carried out to determine the effect ACR has on presynaptic inhibition.

Eight untreated and eight ACR-treated cats were used (30 mg/kg/day for 10 days). On day 11 the animal was anesthetized with ether, respired, the spinal cord transected at C1, and the ether removed. A dorsal laminectomy was performed from L3-S2, the dura opened, and dorsal roots L6 and L7 isolated. L6 was cut distal to where it enters the cord. The sural nerve was exposed on the ipsilateral hindlimb. A stimulating electrode was placed on the sural nerve and recording electrodes were placed on the intact L7 and cut L6 dorsal roots. Input-output relationships were compared in the ACR and control groups by recording the compound action potential across L7 as the input and recording the dorsal root reflex (DRR) evoked in L6 as the output. The relative input necessary to produce a liminal DRR response was defined as the critical input. The voltage required to produce a liminal response on the input was also recorded. Following these studies, the L7 dorsal root was cut distally and split into two rootlets. One rootlet was stimulated at group I strength and the dorsal root potential (DRP) was recorded in the other.

We found that approximately 25-35% of the input was necessary to produce a liminal DRR in control animals. No DRR could be evoked in the ACR-treated cats even with greater than C-fiber stimulation of the sural nerve. The stimulus voltage necessary to produce a liminal input response in the sural nerve was increased at least 10 fold in the ACR-treated animals. The threshold DRP in control animals could be elicited with as little as 0.27±0.054 volts applied to the L7 rootlet. In the ACR-treated animals, the DRP could not be evoked even with the stimulation voltage in excess of 60 volts.

The inability to evoke either a dorsal root reflex or potential suggests that sensory processing in the dorsal horn of the spinal cord, in particular pre-synaptic inhibition is greatly impaired following the administration of acrylamide. Supported by NIH grant NS 18664-02.

293.15 DECREASED SUBSTANCE P (SP) LEVELS FOLLOWING A NOCICEPTIVE STIMULUS IN THE DORSAL HORN OF RATS EXPOSED TO ACRYLAMIDE (ACR). Barry Goldstein, Margaret Kirby, and Norman Holland*. Depts. of Pharmacology and Toxicology and Anatomy, Medical College of Georgia, Augusta, GA 30912.

ACR produces a central-peripheral distal axonopathy. Classically, it is believed that the largest diameter fibers are the most susceptible to the actions of ACR. However, some studies suggest that small diameter fibers are also affected very early in the time course of the axonopathy (Post and Mcleod, 1977; Miller, et. al., 1984).

SP is believed to be involved in the transmission of nociceptive information from small diameter primary afferent fibers. We have previously quantitated normal SP levels and those evoked by an acute nociceptive stimulus by immunohistochemistry in the dorsal horn of the rat (Kantner, et. al., 1985). The purpose of this study was to determine whether chronic administration of ACR would alter resting levels of SP in the spinal cord dorsal horn and those evoked by an acute nociceptive stimulus.

Female rats (225-250 gm) were administered 50 mg/kg ACR for 10 days. On day 11, the animals were anesthetized with urethane and the right hindpaw injected with 0.4 ml of 5% formalin or saline. Sixty minutes after injection with formalin or saline, the rats were killed by perfusion with ice-cold 4% paraformaldehyde in phosphate buffer. The spinal cord was isolated and L5 was removed, postfixed for 90-120 min., frozen in liquid $N_2$, and stored at -70°C until immunohistochemical processing. Immunostaining was done according to a modification of the peroxidase anti-peroxidase (PAP) method of Sternberger. Ten to twelve microdensitometeric readings (1.25 mm aperture, 16x objective) were made of the upper two laminae of the dorsal horn in a minimum of three sections. Gelatin matrices containing known concentrations of SP were sectioned and processed through the PAP procedure so that a standard curve could be generated. Conversions were then made of microdensitometric readings to concentration of SP.

The resting SP levels, i.e. without formalin stimulus, in control and ACR-treated rats were 0.43±0.01 umoles and 0.4±0.02 umoles ($\bar{X}$±SEM), respectively. The SP levels following the nociceptive stimulus were 1.24±0.12 umoles in control and 0.83±0.21 umoles in the ACR-treated group. This represents a 3-fold increase in the SP levels in control rats while the ACR-treated rats only increased 2-fold.

In summary, SP levels appear to be the same in both control and ACR-treated rats prior to a nociceptive stimulus. However, the increase in SP following the formalin injection was partially blocked by ACR treatment. Supported by NS-18664.

294.1 BLOCKADE OF THE AMYGDALOID NUCLEI PARTIALLY REVERSES DOCA-SALT HYPERTENSION. J.E. Szilagyi and J.E. Skinner. Neurophysiology Section, Neurology Department, Baylor College of Medicine, Houston, TX 77030.

Cryoblockade has previously been employed in our laboratory to demonstrate that neuronal activity in projections of the frontocortical-brainstem pathway is a modulator in the maintainance of elevated arterial pressure in DOCA-salt hypertensive rats (Fed Proc 43: 443, 1984; 44: 629, 1985) as well as in the initiation of lethal arrhythmias in the ischemic heart of the psychologically stressed pig (Amer J Physiol 240: H156, 1981). Cryoblockade of the amygdaloid nuclei, which project through the hypothalamus to the brainstem, also prevents stress related arrhythmogenesis (Neurosci Abst 9:1057, 1983). We now report that cryoblockade in the amygdala sufficient to halt synaptic but not axonal activity partially reverses hypertension in the DOCA-salt experimental model. Further cooling results in an additional reduction in arterial blood pressure, possibly due to expansion of the cooling gradient to include more neurons. Heart rate at each cryoblockade temperature was also significantly reduced. These data provide evidence that multiple cerebral systems influence hypertension. These same systems also are known to reduce arrhythmogenesis when blocked and thus provide a neurological explanation for why hypertension and stress are risk factors in arrhythmogenesis.

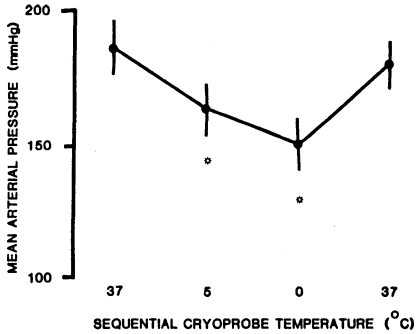


Figure 1: Reduction in mean arterial pressure during sequential cooling and rewarming of the bilateral amygdala cryoprobes. All values are mean ± SEM. * p<0.01.

294.2 HYPOTHALAMIC KNIFE CUTS ATTENUATE VASOPRESSIN-MEDIATED RECOVERY OF BLOOD PRESSURE FOLLOWING HEMORRHAGE. S.L. Bealer. Dept. of Physiology, Univ. Tenn. Ctr. Hlth. Sci., Memphis, TN 38163.

Hemorrhage results in neural and endocrine responses designed to restore blood pressure to normal. Vasopressin secretion is enhanced following hemorrhage, and the pressor effects of vasopressin are critical for recovery of blood pressure. However, the precise neural pathways which mediate this response are not completely understood. The effects of knife cuts posterior to the paraventricular nucleus alone (PVN-cut), or to both the paraventricular and supraoptic nuclei (PVN/SON-cut), on vasopressin dependent recovery of blood pressure following hemorrhage were tested in the rat. Animals received knife cuts or control surgery and were implanted with femoral artery and jugular vein catheters 2-3 weeks later.

The conscious, unrestrained rats were hemorrhaged a volume of blood equivalent to 1.8% body weight from the femoral artery. Blood pressure was continuously monitored through the arterial catheter for 30 min with no treatment. Rats then received an intravenous injection of a specific antagonist to the pressor action of vasopressin, and blood pressure was monitored for an additional 30 min. At this time, an intravenous infusion of the competitive antagonist for angiotensin II, saralasin, was initiated and continued for 15 min. Similar treatments were conducted in knife-cut and control-operated animals which were not hemorrhaged.

Prehemorrhage blood pressure was similar in all groups of rats (Cont, 100 ± 4 mm Hg; PVN-cut, 111 ± 4 mmHg; PVN/SON-cut, 102 ± 3 mmHg). In addition, the immediate post-hemorrhage decrease in blood pressure and the initial restoration of blood pressure 5 min following hemorrhage was not different between both groups of knife-cut rats and control-operated animals. However, 10 min after hemorrhage, blood pressure in control-operated and PVN-cut animals was not significantly different than pre-hemorrhage levels, while blood pressure in PVN/SON-cut animals remained significantly depressed. This relationship continued throughout the observation period. Injection of the specific antagonist of the pressor effects of vasopressin produced equivalent decreases in blood pressure 30 min following injection in control-operated (-16 ± 3 mmHg) and PVN-cut rats (-15 ± 2 mmHg), but did not alter blood pressure in PVN/SON rats (-1 ± 4 mmHg). Infusion of saralasin resulted in equivalent decreases in blood pressure in all groups of animals. These data show that in rats with knife cuts confined to the area posterior to the paraventricular nuclei, vasopressin contributed to the restoration of blood pressure following hemorrhage. However, when knife cuts extended into the ventral hypothalamus, the contribution of vasopressin was eliminated.
(Supported by USPHS Grant HL-25877, and USPHS RCDA HL-01237.)

294.3 ALTERED NORADRENERGIC ACTIVITY IN SPECIFIC HYPOTHALAMIC AREAS OF DAHL SALT-SENSITIVE RATS. K.P. Patel*, J.D. Peuler*, C.A. Whiteis*, B.J. Pardini, D.D. Lund and P.G. Schmid. V. A. Med. center., Cardiovascular center., and Dept. Int. Med., Univ. of Iowa, Iowa City, IA. 52240.

Central neurogenic mechanisms have been implicated in Dahl hypertensive rats. Lesions of anteromedial hypothalamus, paraventricular nuclei, or anteroventral third ventricle (AV3V) region have been shown to normalize arterial pressure in Dahl salt-sensitive rats. In the present study we examined the noradrenergic activity in various discrete hypothalamic areas postulated to be involved in the control of arterial pressure of 10-week old, female, Dahl salt-sensitive rats fed low (0.13%) or high (8.0%) salt (NaCl) diets (SL, SH respectively) for six weeks postweaning. The Dahl salt-resistant rats on similar regimen of low and high salt diets (RL, RH respectively) were used as controls. Blood pressure was elevated in SH group compared to RL, RH and SL groups (P<0.05). An index of norepinephrine (NE) turnover was determined by measuring the decline in tissue NE concentration 8 hours after administration of alpha-methyl-p-tyrosine (300 mg/kg, Q4hr, i.p.) in a group of animals from each of the four groups: RL, RH, SL and SH (n=5-8 per group). The data are expressed as a percent of mean initial value obtained from animals killed at zero time in each group (n=6-9 per group) (mean±S.E.) . A greater percent of NE remaining after inhibition of synthesis indicates a decreased turnover of NE in that particular tissue.

| | MPO | PO | PVN | SON | AH | PH |
|---|---|---|---|---|---|---|
| RL | 81±14 | 85±15 | 119±27 | 102±15 | 86±20 * | 49±7 * |
| RH | 69±10 | 82±11 | 78±10 | 71±14 | 82±9 * | 65±5 * |
| SL | 109±31 | 63±12 | 88±22 | 64±12 | 128±26 | 80±27 |
| SH | 59±21 | 55±12 | 79±17 | 51±18 | 152±26 | 123±19 |
| Strain diff. | | R>S | | R>S | S>R | S>R |

MPO = median preoptic area, PO = preoptic area, PVN = paraventricular nucleus, SON = supraoptic nucleus, AH = anterior hypothalamus, PH = posterior hypothalamus. * = p<0.05 vs SH.

There was increased turnover of NE in the preoptic area and supraoptic nucleus of salt-sensitive strain compared to salt-resistant strain. In contrast, NE turnover was decreased in anterior and posterior hypothalamus of the salt-sensitive strain compared to the resistant-strain. Generally SH rats had significantly different turnover of NE compared to the R-strain. These results indicate that there are genetic differences in the noradrenergic activity of hypothalamic areas in Dahl rats, which may be related to the increased arterial pressure observed in the SH group. Supported by Veterans Administration, HL-20768 and HL14388.

294.4 OPIOID μ-RECEPTORS AND CARDIOVASCULAR CONTROL: EFFECTS ON CARDIAC OUXPUT AND REGIONAL BLOOD FLOW. A.L. Siren and G. Feuerstein. Dept. of Neurology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

We have previously shown that centrally administered D-ALA$^2$-MePhe$^4$-Gly$^5$-ol-enkephalin (DAGO), a selective μ-opiate receptor agonist, induces changes in blood pressure and heart rate in conscious rats. The aim of the present study was to investigate the effects of DAGO, microinjected into the medial preoptic nucleus of hypothalamus (POM), on cardiac output and regional blood flow in the conscious rat (n=6-11). The animals were chronically instrumented for cardiac index (CI, thermodilution, Cardiomax) or blood flow (BF, directional pulsed Doppler technique) measurements. BF in hindquarters (HQ), mesenteric (M) and renal (R) arteries and the blood pressure and heart rate were continuously recorded. Microinjections of DAGO (1 or 10 nmol/rat) into the POM increased the mean arterial pressure (MAP) and pulse pressure (PP) in a dose-related manner to a maximum of +17±3 mmHg (p<0.001), respectively by the higher dose of DAGO. Central injections of DAGO induced a biphasic heart rate response: after an initial bradycardic effect (-74±17 bpm, p<0.001) the heart rate increased up to +106±13 bpm (p<0.001) 2 h after the drug administration. Concomitantly with the pressor and bradycardic responses, DAGO (10 nmol/rat) elicited a long-lasting rise of CI (max +18±5%, p<0.01) but had no significant effect on the calculated total peripheral resistance index (TPRI). Microinjections of DAGO into the POM increased the HQ BF and decreased the BF in M and R. The maximum increase in HQ BF after the 10 nmol/rat dose was +20±5% (p<0.001). The BF in M initially sharply decreased reaching its maximum (-30±4%, p<0.001) at about 3 min after the drug injection and remained impaired during the whole observation period of 2 h. The BF in R gradually decreased with the maximum drop (-22±5%, p<0.05) becoming apparent about 30 min after the injection. The calculated vascular resistance (VR) briefly decreased in HQ and dose-dependently increased in M and R. The maximum changes in VR after the 10 nmol dose of DAGO were: -10±3% (p<0.001) in HQ, +67±7% (p<0.001) in M and +51±12% (p<0.001) in R. The results indicate that the pressor response to centrally injected DAGO is due to its action on the CI. Since the increase in CI was accompanied by bradycardia, a positive inotropic effect seems to underly the increase in CI produced by DAGO. The lack of effect of DAGO on TPRI, may reflect the opposite action of DAGO on HQ and M BF and VR. This pattern of the DAGO-induced changes in organ BF suggests an involvement of the sympathetic nervous system in the cardiovascular effects of opioids, and a role of μ-receptors in the POM in redistribution of BF in peripheral organs.

294.5 ROLE OF CENTRAL OPIOID RECEPTORS IN BAROREFLEX CONTROL OF SYMPATHETIC AND CARDIOVASCULAR FUNCTION. F.J. Gordon* (SPON: R.F. Kibler) Dept. Pharm., Emory Univ. Sch. of Med., Atlanta, GA 30322.

These studies examined the influence of central opioid receptor activation on baroreflex regulation of mean arterial pressure (MAP), heart rate (HR), and sympathetic nerve activity (SNA). Rats were anesthetized with urethane, paralyzed, and artificially ventilated. The femoral artery and vein were catheterized for measurement of MAP and HR, and peripheral drug injection. SNA was recorded from the lumbar sympathetic chain. Arterial baroreceptor reflexes were provoked by electrical stimulation (5V, 2 msec) of the aortic nerve (AN) at graded frequencies (1.25 - 20 Hz) after all other arterial baroreceptor afferents had been destroyed. Baroreflex response curves were constructed following intracisternal (IC) administration of saline vehicle (5 µl), after IC injection of the relatively selective mu and delta opioid receptor agonists D-Ala$^2$, MePhe$^4$, Gly-ol$^5$ enkephalin (DAGO) or D-Ala$^2$, D-Leu$^5$ enkephalin (DADLE), and again after i.v. naloxone (0.1 and 0.5 mg/kg). Six groups of rats (n=6-7) were studied. Separate groups received IC DAGO (0.01, 0.1, 1.0 nmole), IC DADLE (1.0 nmole), i.v. DAGO (1.0 nmole), or i.v. naloxone (0.1 and 1.0 mg/kg). Graded reductions in MAP (15-60 mmHg), HR (20-130 bpm), and SNA (15-65%) produced by AN stimulation were not significantly different between the 6 groups after IC saline. IC injection of opioid peptides did not elicit any consistent pattern of cardiovascular or SNA change. Intravenous naloxone alone was also without effect. Reflex reductions in MAP, HR, and SNA elicited by AN stimulation were attenuated in a dose-dependent fashion following IC DAGO. The largest impairment (60-80%) was observed at low frequencies of AN stimulation (1.25-5.0 Hz), and became progressively less marked as the frequency of AN stimulation was increased. At 10-20 Hz only a 10-30% attenuation of baroreflex responses was observed. The inhibitory action of IC opioids was completely reversed by i.v. naloxone. Neither i.v. naloxone alone or i.v. DAGO alone had any effect on baroreflex responses. IC injection of the delta receptor agonist DADLE also attenuated baroreflex responses. The potency of this peptide was 10-100-fold less than that of DAGO, and baroreflex inhibition was completely reversed by the lowest dose of i.v. naloxone (0.1 mg/kg). These results indicate that: 1) stimulation of central mu receptors with DAGO significantly impairs arterial baroreflex control of sympathetic and cardiovascular function, 2) these effects are most marked at low levels of baroreceptor activation, 3) baroreflex impairment produced by IC DADLE was probably mediated by mu rather than delta opioid receptors. No evidence was obtained to suggest a role for endogenous opioid modulation of arterial baroreceptor reflexes since i.v. naloxone was without effect. (Supported by NIH-HL30537 and a PMA Foundation Research Starter Grant)

294.6 PREVENTION OF THE DEVELOPMENT OF ADULT ESSENTIAL HYPERTENSION BY SELECTIVE TOXICITY OF GLUTAMATE-SENSITIVE CARDIOVASCULAR CIRCUITRY AT CIRCUMVENTRICULAR ORGANS OF THE ADOLESCENT SPONTANEOUSLY HYPERTENSIVE RAT. D.K. Hartle and P.V. Krishnamurti*. Dept. of Pharmacology, Emory Univ. Sch. of Med., Atlanta, GA 30322.

The pattern of neural toxicity produced by peripheral administration of large doses of monosodium glutamate (MSG) is limited to glutamate-sensitive soma and dendrites within the circumventricular organs (CVOs). Other regions of the brain are protected by the blood-brain barrier. Four CVOs have been implicated in blood pressure regulation. We recently reported that MSG toxicity reverses essential hypertension in the adult Spontaneously Hypertensive Rat (SHR), but the same treatment produces no hypotension in the adult Wistar Kyoto (normotensive) Rat (WKY). The present studies were done to determine if MSG toxicity in either the neonatal or adolescent period could prevent the development of essential hypertension in the SHR. SHR pups were treated with 4 mg/g MSG or isotonic saline s.c. at 4 days of age (n=8, each group). When the rats were 14 weeks of age, direct blood pressures were recorded by arterial catheter while the animals were conscious and unrestrained. Blood pressure of the MSG-treated animals were not significantly different from the control group. Because hypertension is not expressed in the SHR until the adolescent period, a second experiment was conducted to determine if MSG toxicity in the very early phase of hypertension (5-7 weeks) would prevent the development of adult essential hypertension. Eleven SHRs were treated with 8 mg/g MSG s.c. at 5 weeks and again at 7 weeks of age. A group of eight SHRs were injected with saline at these ages and served as the control group. At 14 weeks of age, direct arterial pressures were recorded in the conscious and unrestrained rats. In contrast to neonatal treatment, MSG treatment during adolescence produced no growth impairment in the SHR. Mean arterial blood pressure was significantly lower in the MSG-treatment group, 120 + 5 mmHg vs 190 + 6 mmHg in the saline treatment group ($p < .001$). The mean blood pressure of the group treated with MSG during adolescence was not significantly different at 14 weeks from those of either 14 week old WKY rats or SHR rats whose hypertension was reversed by MSG treatment at 14 weeks of age. These data suggest that the development of essential hypertension in the SHR is dependent upon the integrity of glutamate-sensitive elements of CVOs. The fact that MSG toxicity in the neonatal period is without effect on the development of hypertension suggests that the specific glutamate-sensitive CVO cardiovascular circuitry involved in the pathogenesis of SHR hypertension is not present in the neonate, but appears later in development. Supported by AHA, Nat'l, AHA, GA, Emory Univ. Res. Fund and PHS#T35-HL30706-06A1.

294.7 ROLE OF THE SYMPATHETIC NERVOUS SYSTEM IN HIGH SODIUM RENAL HYPERTENSION DURING CHRONIC VASCULAR VASOPRESSIN RECEPTOR BLOCKADE. C. Hinojosa* and J.R. Haywood. Dept. of Pharmacology, Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX 78284.

The sympathetic nervous system (SNS) and arginine-vasopressin (AVP) have been shown to contribute to the development of several models of sodium dependent hypertension. Our previous studies demonstrated that the SNS and AVP maintain the elevated blood pressure (BP) in high sodium fed one kidney, figure-8 wrap hypertensive rats. The purpose of this study was to determine if the pressor effect of AVP was necessary for the development of the hypertension by chronic subcutaneous administration of a vascular AVP antagonist, d($CH_2$)$_5$Tyr(Me)AVP, (AA) via an osmotic minipump. In addition, the contribution of the SNS to BP was determined in these animals. One group of high sodium fed rats was treated with AA and the other group received vehicle (V). Two days later, the animals underwent unilateral nephrectomy combined with renal figure-8 wrap (W) or sham (S) surgery which was unilateral nephrectomy only. BP and heart rate were monitored one day before and 5 days after renal surgery. In addition, AVP receptor blockade was confirmed periodically by a 80% reduction of the pressor response to a bolus injection of 10 mU/kg AVP. Baseline BPs are shown below.

| | CONTROL | DAY 1 | DAY 3 | DAY 5 |
|---|---|---|---|---|
| V + S | 124 ± 4 | 119 ± 4 | 117 ± 4 | 123 ± 4 |
| AA + S | 120 ± 3 | 115 ± 3 | 114 ± 3 | 118 ± 3 |
| V + W | 119 ± 1 | 132 ± 6 | 138 ± 5 | 138 ± 6 |
| AA + W | 121 ± 3 | 141 ± 3 | 140 ± 4 | 147 ± 4 |

On day 5 after renal surgery all animals were given an i.v. injection of 25 mg/kg hexmethonium and 0.2 mg/kg atropine to produce ganglionic blockade (GB). In V + S and V + W rats, GB decreased BP by 38 ± 2 and 38 ± 3 mmHg, respectively. However, a subsequent bolus injection of AA caused a greater fall in BP in V + W rats (-30 ± 4 mmHg) than in V + S rats (-13 ± 4 mmHg), such that the final BPs were equalized to 75 ± 4 and 71 ± 3 mmHg, respectively. In AA treated S and W rats, GB also caused similar depressor effects of -52 ± 3 and -60 ± 7 mmHg, respectively. An additional bolus injection of AA had no effect on BP in either group, such that the final BP in AA + W rats (90 ± 6 mmHg) was greater than in AA + S rats (62 ± 3 mmHg). The difference in BP was not angiotensin II-dependent since captopril caused similar depressor effects in both groups. These results indicate that AA treatment did not affect baseline BP in S rats and did not prevent or attenuate the development of the hypertension in W rats. However, the mechanism of the elevated BP is not through an activation of the SNS or other neurohumoral mechanisms indicating that volume or other factors may be involved.

294.8 ANGIOTENSIN II NEURONAL PERIKARYA IN HUMAN CIRCUMVENTRICULAR ORGANS, S. Landas*, R.L. Schelper*, L.D. Wilkin, L.D. Mitchell, A.K. Johnson. Univ. of Iowa Coll. of Medicine and Cardiovascular Center, Iowa City, IA 52242.

An important role for the central nervous system in animal models of hypertension is widely accepted. Brain angiotensin II (AII) is a major component of many central hypertensive theories. In rats, AII systems have been shown to be involved in control of blood volume as well as the generation of certain types of pressor reflexes. Chronic administration of AII antogonists has been shown to ameliorate the development of hypertension in spontaneously hypertensive rats (Phillips et al., Physiologist, 18:350, 1975). Some of these AII systems have been localized to forebrain circumventricular organs (CVO) and shown to project to nuclei in the anterior hypothalamus (Lind et al., Brain Res., 321:209, 1984; Mitchell et al., Neurosci. Abstr., 10:480, 1984). In a preceding study, human Golgi-stained cells and rat AII containing neurons were compared and found to have similar dimensions and morphology (Mitchell et al., Fed Proc, 44:1199, 1985). However, AII neuronal systems have not yet been demonstrated in humans. It is crucial to show homology between human and animal systems if information derived from animal models of hypertension is to be applied to the human disease state.

In the present study, we examined AII immunoreactivity in human brain collected at autopsy using the unlabeled antibody-peroxidase technique. Previous observations of AII neuronal processes or fibers in human brain were confirmed. Additionally, AII immunoreactive perikarya were found within the subfornical organ, the median preoptic nucleus, and the lamina supraoptica (organum vasculosum of the lamina terminalis). These cells had a "primitive" appearance with ovoid to nearly spherical somata 10-15 um in diameter, one or two short dendritic processes, and a long axon. These AII neurons were frequently located in close proximity to blood vessels in the subfornical organ and the lamina supraoptica. AII immunoreactivity was also found within some magnocellular neurons of the paraventricular nucleus of the hypothalamus. These findings stand as important observations in the correlation of animal AII data with human physiology and disease. (Supported in part by NIH grant HL32192 to L.D.W.)

294.9 SALT APPETITE IN RATS AFTER PERIPHERAL PHYSIOLOGICAL DOSES OF ANGIOTENSIN. A.D. Dalhouse, O. Brooks*, A. Cook*. Dept. Psy., Jackson State University, Jackson, MS 39217. H.G. Langford* Dept. Med., Univ. of Miss. Med. Ctr., Jackson, MS 39216.

Sodium (Na) deprived mammals develop increased Na appetite and increased renal secretion of renin which causes the cleavage of angiotensin I (A I) from its parent protein. A I in the presence of converting enzyme is transformed into angiotensin II (A II) and A II increases the adrenal NA retaining steroid, aldosterone (aldo). Increasing doses of desoxycorticosterone acetate (DOCA) to adrenalectomized rats produce a V-shaped NA intake curve with decreasing Na consumption until a nadir is reached, then increasing consumption (Fregly & Waters, Physiol. Behav., 1, 65, 1966). These data are compatible with the assumption that both A II and aldo can increase Na consumption. The untreated rats would have had high A II levels which should stimulate Na appetite. With increasing amounts of aldo Na retention should occur, renin secretion should decrease, and Na appetite decrease. With increasing amounts of aldo, the increasing Na appetite can be attributed to the mineralocorticoid alone, for renin secretion should be well suppressed. The experiments reported here were designed on the assumption that an experimental design which started with A II fully suppressed would be optimum to test the effects of A II on Na appetite. It was felt desirable to use the minimum amount of mineralocorticoid compatible with full renin suppression to avoid confusion with mineralocorticoid elicited Na appetite.

Two experiments were conducted in which adrenalectomized male Sprague-Dawley rats (200g) were maintained ad libitum on distilled water, 3% saline (cafateria style) and Na free food. In Experiment I, 45 rats were given 100, 200, 400, 800, or 1000 ug/kg/day im. DOCA for 5 days to determine the dose that would produce the lowest voluntary Na intake, and 800 ug/kg/day was found to produce the nadir in Na intake ($p < 0.05$). In Experiment II, 40 rats were placed ad libitum on distilled water, saline and Na free food as described above, maintained on 800 ug/kg/day DOCA, and infused with 5, 25, 100 ug/kg/day A II or 0.9% saline for 5 days. The three A II groups showed significant percent changes in their Na intake above pre-A II levels and the saline group ($p < 0.02$), but no differences between each other ($p > 0.05$). These results were interpreted to demonstrate the production of Na appetite in rats by peripheral administration of physiological doses of A II.

Supported by NIH/MBRS 5-S06-RR08047-11 and NIH RO1-HL24369.

294.10 THE ROLE OF THE RENIN-ANGIOTENSIN SYSTEM IN THE MAINTENANCE OF ARTERIAL PRESSURE IN RATS WITH LESIONS OF THE LATERAL PARABRACHIAL AND KÖLLIKER-FUSE NUCLEI. J.W. Hubbard, R.A. Buchholz, T.K. Keeton*, and M.A. Nathan. University of Texas Health Science Center, San Antonio, Texas 78284.

Previous studies have shown that the parabrachial nuclei of the dorsolateral pons project to and receive projections from structures in the central nervous system which regulate fluid-electrolyte balance and arterial pressure (Fuleviler and Saper, Brain Res. Rev. 7:229-259, 1984). However, the role of these nuclei in the neurohumoral regulation of arterial pressure is unknown. Therefore, we examined the effects of bilateral electrolytic lesions of the lateral parabrachial and Kölliker-Fuse nuclei (LPB-KF) on plasma renin activity (PRA), plasma norepinephrine (NE) and epinephrine (E) concentrations, mean arterial pressure (MAP) and heart rate (HR) in conscious rats.

Male Long Evans rats were anesthetized with sodium pentobarbital (50 mg/kg, i.v.) and bilateral electrolytic lesions were made in the LBP-KF by passing a DC anodal current (0.8 mA) through a Teflon-coated tungsten electrode (0.18 mm, o.d. with a 0.2 mm tip exposure) for 15 seconds. Sham-lesioned (control) animals received similar treatment except that no lesions were made in LPB-KF. Cannulas were implanted in the left femoral artery and vein for the measurement of arterial pressure and the administration of drugs, respectively. Cardiovascular and neuroendocrine measurements were then made 3-7 days following the lesions.

We found that lesions of the LPB-KF did not alter baseline MAP, HR, or plasma NE and E concentrations compared to the control rats. However, the basal PRA in rats with LPB-KF lesions were 6.5 times higher than the basal PRA in sham-lesioned control rats. This increase in PRA was associated with normal plasma sodium and potassium concentrations and slightly elevated plasma osmolality.

| *$p < 0.05$ | | MAP (mm Hg) | HR (bpm) | PRA (ng AI/ml/hr) | NE (pg/ml) | E (pg/ml) |
|---|---|---|---|---|---|---|
| LPB-KF | (4) | 122±4 | 384±6 | 43.3±11.1* | 180±22 | 88±35 |
| Control | (4) | 116±5 | 355±9 | 6.7±1.9 | 143±32 | 63±28 |

$\beta_1$-Adrenoceptor blockade with atenolol (1 mg/kg, i.v.) normalized PRA in the LPB-KF rats. Sequential blockade with captopril (10 mg/kg, i.v.), vasopressin antagonist $d(CH_2)_5Tyr(Me)AVP$ (15 µg/kg, i.v.), and chlorisondamine ( 3 mg/kg, i.v.) revealed that the renin-angiotensin system plays in important role in the maintenance of resting MAP in rats with LPB-KF lesions. Supported by NIH grant HL33635 and AHA, Tx. Affil.

294.11 DIFFERENTIAL EFFECTS OF STIMULATION OF NUCLEUS AMBIGUUS ON ATRIAL AND VENTRICULAR RATES. M.E. Thompson*, G. Felsten, J. Yavorsky*, and B.H. Natelson. VA Med. Ctr. and New Jersey Med. School, East Orange, N.J. 07019.

The vagus is the primary efferent pathway for a variety of reflexes affecting heart rate and rhythmicity. The origin of vagal preganglionic neurons in the area of nucleus ambiguus (NA) has been recently established by neuroanatomical tracing methods. However, it is unclear which of these cells affect the SA node and which affect the AV node. Electrical and chemical stimulation in this area has been shown to produce bradycardia. Whether this represents both chronotropic (CE) and dromotropic (DE) effects is unclear. Therefore, the present study sought to determine whether stimulation at different sites within NA would produce differential effects on atrial (AR) and ventricular (VR) rates. Acute experiments were performed on open-chested male mongrel dogs which were anesthetized (pentothal), and respired. A pacing electrode was placed on the left atrial appendage and catheters were placed in the carotid artery and jugular vein. This latter catheter provided an atrial lead for the ECG. An average of 10 penetrations per side was used to explore the rostral-caudal organization of the NA and the area ventrolateral to it in .5-1.0 mm steps. Electrical stimulation with 50 pps, .1 ms square wave pulses varying in intensity from 100-300 uA was delivered in 5 second trains as the electrode was lowered in .2-.5 mm steps. Pacing was performed on some trials to clarify the occurrance of AV block. When pacing was used, the rate was adjusted to be as close to the unpaced rate as possible while maintaining control.

Stimulation in the area of the NA and lateral to it produced bradycardia and bradyarrhythmias without altering blood pressure more than 15 mmHg. Stimulation of either the left or right NA produced slowing of both VR and AR. However, slowing of the AR (i.e.,CE) was greatest with right NA stimulation (AR=36; VR=24; paced VR=144) while slowing of the VR (i.e.,DE) was predominant with left side stimulation (AR=72; VR=24; paced VR=60). The presence of AV block was evident from dropped QRS complexes with and without pacing. Futhermore, there was a rostral-caudal organization of AV dissociation with posterior sites producing greater AV block than anterior sites. From this evidence we conclude that the vagal preganglionic neurons controlling SA and AV nodes arise from different areas within NA. Control of AV conduction seems to predominate in the left and posterior areas of NA while the anterior and right side is predominantly chronotropic.
(Supported by HL 24498)

294.12 NEURONS IN NUCLEUS AMBIGUUS RESPOND TO CHANGES IN BLOOD PRESSURE. G. Felsten, M.E. Thompson*, J.A. Cook* & B.H. Natelson. VA Med. Ctr. & New Jersey Medical School, East Orange, N.J. 07019.

Because recent work suggests that the vagal preganglionic neurons mediating the baroreflex in cat and dog are located in or near nucleus ambiguus (NA), and not in the dorsal motor nucleus of the vagus, as previously thought, we investigated the responses of neurons in the region of NA, while manipulating arterial blood pressure (BP) nonpharmacologically. Sodium pentothal anesthetized dogs, were thoracotomized to permit the placement of inflatable silastic occlusion cuffs around the descending aorta (DA) and inferior vena cava (IVC), to increase and decrease arterial blood pressure, respectively. BP increases averaged 80 mm Hg, while BP decreases averaged 60 mm Hg. The dogs remained open-chested and were artificially respired. Stereotaxically placed stainless steel electrodes were used to record single or multiple unit activity from cells in or near nucleus ambiguus. For each unit or small group of neurons isolated, we recorded ECG, BP and neural activity with no manipulation of BP, and with BP increased and decreased 4 times in semi-random order.

Most neurons had spontaneous activity rates of less than 5 spikes/sec and some were completely quiet until blood pressure was manipulated. Brainstem neurons sensitive to changes in BP could be divided into 2 subgroups. 77% of these neurons (Group 1) responded with increased firing as BP increased during inflation of the DA cuff. Heart rate decreased from 5% to 40% following the increase in neural activity. The firing rate of these cells did not change as BP decreased due to inflation of the IVC cuff, but increased as BP increased when the IVC cuff was deflated. The other 23% of BP sensitive neurons (Group 2) had clear respiratory rhythms with spontaneous rates cycling from as low as 0 to as high as 50 spikes/sec. The activity of Group 2 neurons was suppressed during increases in BP and enhanced during BP decreases. These two groups differed not only in regard to respiratory properties, but also in that Group 2 neurons responded to BP increases and decreases, while Group 1 neurons responded only to BP increases. These differences may be due to input from different populations of BP receptors, with Group 1 neurons affected by stimulation of arterial and left ventricular receptors, and Group 2 neurons affected by stimulation of any combination of arterial, left ventricular, atrial or pulmonary receptors.

Our data indicate that the firing rates of some neurons in the area of nucleus ambiguus in the dog are altered by changes in BP, and that these neural response changes precede changes in heart rate. In conjunction with anatomical and physiological work indicating that NA is the site of origin of vagal preganglionic fibers to the heart, we conclude that these neurons participate in mediating the baroreceptor reflex. (Supported by HL 24498).

294.13 NEUROGENIC REGULATION OF ARTERIAL PRESSURE LABILITY IN RATS FOLLOWING SINO-AORTIC DEAFFERENTATION (SAD). R.H. Alper and M.J. Brody. Dept. of Pharmacology and CV Center, University of Iowa, Iowa City, IA 52242.

Aortic and carotid baroreceptors are involved in minute-to-minute regulation of arterial pressure. Baroreceptor deafferentation causes only relatively acute hypertension, but arterial pressure lability is increased chronically. Increased sympathetic tone accounts for the acute hypertensive phase, but mechanisms generating lability are unclear. These studies were to investigate neurogenic regulation of arterial pressure lability in rats following chronic SAD.

SAD or sham surgery was performed on male rats 10-14 days prior to experiments. Catheters were placed in the femoral artery and vein for pressure measurement and drug administration, respectively, in conscious, unrestrained rats. Animals showing impaired baroreflex function (<20% of control bradycardia in response to phenylephrine) were considered as SAD. Mean arterial pressure (MAP) and labiltiy (the standard deviation of the MAP) were determined for a 1 hr control period. Drugs were then injected and MAP was recorded for an additional 1 hr. In all experiments, MAP was the same in intact and SAD rats prior to drug treatment. Labiltiy, on the other hand, was always greater in rats following chronic SAD.

Ganglionic blockade by chlorisondamine (2.5 mg/kg) caused equivalent, sustained hypotension in all rats. Lability was increased in intact and decreased in SAD rats to values between those observed in untreated intact and SAD rats.

Yohimbine (2 mg/kg) did not alter MAP or lability in any rats. This dose was not selective for $\alpha_2$-adrenergic receptors as it partially attenuated the pressor response to phenylephrine.

The selective $\alpha_1$-adrenergic receptor antagonist prazosin (0.1 mg/kg) blocked the pressor response to phenylephrine and slightly decreased both MAP and lability in all rats. Lability was still greater in SAD than intact animals.

The simultaneous administration of prazosin and yohimbine (in the same doses used above) caused marked hypotension for 30 minutes. Arterial pressure rose during the next 30 minutes, but did not return to control values. Combined $\alpha$-receptor blockade increased lability in intact rats but decreased lability in SAD rats, similar to what was observed after chlorisondamine.

These data suggest that arterial pressure lability in rats following chronic SAD is not generated through selective activation of $\alpha_1$- or $\alpha_2$-adrenergic receptors. Neurogenic mechanisms appear to contribute to, but may not totally account for, fluctuations in arterial pressure characteristic of baroreflex deficient rats. (Supported by HLB-14338).

294.14 THE DEVELOPMENT OF THE RENAL $\alpha_2$-ADRENERGIC RECEPTOR IN THE RAT. B. Sripanidkulchai, R. Dawson and J.M. Wyss. Department of Cell Biology and Anatomy, University of Alabama at Birmingham, AL 35294.

The renal $\alpha_2$-adrenergic receptor in the rat has two affinity binding sites. In the present study the development of these two sites was investigated using agonist [($^3$H)-para-amino clonidine, PAC] binding to crude membrane preparation in Sprague-Dawley rats from day of birth through adulthood.

Scatchard analysis of ($^3$H)-PAC binding to renal $\alpha_2$-adrenergic receptors on the day of birth reveals a single binding site with a KD of 0.9 nM and Bmax of 15 fmoles/mg protein. In contrast, analysis of kidneys from day 3 rats indicates that 2 sites exist at this stage, one with a KD of 0.9 nM and Bmax of 16 fmoles/mg protein (the high affinity site) and the other with a KD of 8 nM and Bmax of 55 fmoles/mg protein (the low affinity site). The absolute number of both sites increases during the next several days with the Bmax nearing adult levels around day 7 for the high affinity site (32 fmoles/mg protein) and day 30 (132 fmoles/mg protein) for the low affinity site. The KD of each site remains stable throughout this period. This suggests that the development of the high affinity $\alpha_2$-adrenergic receptor site predates that of the low affinity site, but the affinity of the sites remain unchanged throughout development.

To determine the possible interaction between development of the sympathetic nervous system and the $\alpha_2$-adrenergic receptor in the kidney, the time course of the renal sympathetic innervation has been characterized by both HPLC biochemical determination of renal norepinephrine content and morphological analysis of tyrosine hydroxylase (TH) positive axons. The results indicate that renal norepinephrine is present at the day of birth (2.42 ng/kidney) and the level increases gradually to adulthood. TH positive fibers are present on the day of birth and increase throughout development. These results indicate that the renal sympathetic innervation is present at birth and therefore could provide the biological signal which induces the appearance of the low affinity renal $\alpha_2$-adrenergic receptor.

294.15 ONTOGENY OF RENAL ADRENERGIC RECEPTOR SITES IN INBRED DAHL HYPERTENSION-SENSITIVE (DS/JR) AND -RESISTANT (DR/JR) RATS. J.A. McCaughran, Jr., C.J. Juno*, and E. O'Malley*. Dept. Psychiatry and Behavioral Science, SUNY at Stony Brook, New York, 11794.

The DS/JR rat develops severe elevations in BP when exposed to hypertensinogenic stimuli. The DR/JR rat remains normotensive when exposed to the same stimuli. Although the mechanisms responsible for the development and maintenance of hypertension have not been determined, there is compelling evidence to suggest that renal sympathetic factors participate. The renal proximal and distal convoluted tubules possess a high density of $\alpha_2$-adrenoceptors. The activation of these has been shown to influence sodium reabsorption and renin release. In the mature rat, studies have found that the density of $\alpha_2$-adrenoceptors is greater in the DS/JR than the DR/JR strain, regardless of the blood pressure. These differences have been related to alterations in natriuresis and renin release, both of which distinguish the DS/JR from DR/JR strain. The ontogeny of these distinguishing characteristics has not been examined. In this study, the $\alpha_2$ antagonist $^3$H-rauwolscine was used to assess the density of renal $\alpha_2$-adrenoceptors in the DS/JR and DR/JR strain at 5, 15, 25, and 50 days of age. In contrast to the adult, the density of adrenoceptors was lower in the DS/JR strain than the DR/JR strain at 5 days of age. The density of adrenoceptors was approximately 10-15% of adult values in the DS/JR strain and 20-25% of adult values in the DR/JR strain. The dissociation constants (Kds) were similar between the strains but lower than adults in both strains. At 15 days of age, the density in the DS/JR and DR/JR strains was approximately 30-35% and 48-53% of adult values, respectively. The Kds approached adult values (i.e., approximately 2.00 nM) and were similar between the strains. By 25 days of age, the density of adrenoceptors was greater in the DS/JR strain than the DR/JR strain. Although the density in the DR/JR strain was similar to adult values at this age, the density of adrenoceptors in the DS/JR strain continued to increase until 50 days of age. The results of this study indicate that the rate of development of $\alpha_2$-adrenoceptors differs between the DS/JR and DR/JR strains. The fact that the density of sites continues to increase to 50 days of age in the DS/JR strain suggests that the density of sites observed in the mature DS/JR rat may in part be due to secondary factors and not solely to the genetic predisposition to hypertension.

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294.16 RENAL DENERVATION CAN PREVENT STRESS-INDUCED HYPERTENSION IN THE BORDERLINE HYPERTENSIVE RAT (BHR). J.E. Lawler, R.H. Cox, B.J. Sanders,* V.P. Mitchell,* and P.G. Baer.* University of Tennessee, Knoxville, 37996, and UTCHS, Memphis.

The F1 cross between the spontaneously hypertensive rat and the Wistar-Kyoto rat develops spontaneous borderline hypertension, and is called the BHR. This animal develops permanent and severe hypertension when exposed to conflict schedules lasting 15 weeks. The purpose of this study was to determine if renal denervation done at two different time periods after exposure to chronic stress could prevent stress-induced hypertension. Thirty-two male BHR were exposed to an uncontrollable cutaneous electric shock schedule for either 5 or 11 weeks. Half of the animals in each group received sham surgery, while the other half received bilateral renal nerve denervation. Tail cuff arterial blood pressures were recorded weekly in all animals. After 5 wks of stress, the systolic blood pressure of BHR was 169 mm Hg. Renal denervation prevented any further rise in blood pressure, since after 10 additional weeks of stress, the blood pressure of denervated BHR was still under 170 mm Hg (164±5 mm Hg), while in sham animals it was over 180 mm Hg (183±6 mm Hg). After 11 wks of stress, the systolic blood pressure of BHR was 179±7 mm Hg. Although denervation temporarily lowered the blood pressure of this group to approximately 160±4 mm Hg, it soon recovered to levels of the sham animals. These data are consistent with the hypothesis that the renal nerves are a necessary component to the development of stress-induced hypertension, since elimination of these nerves during a critical period prevents further development of hypertension. Once stress-induced hypertension has developed, renal denervation has no permanent protective effect on blood pressure. (Supported by HL-19680 and by HL-01395)

294.17 FURTHER ANALYSIS OF THE EFFECT OF DORSAL RHIZOTOMY ON GOLDBLATT HYPERTENSION. N. Aboukarsh, W. Sripairojthikoon and J.M. Wyss, Department of Cell Biology and Anatomy, University of Alabama at Birmingham, AL 352 94.

In past studies it has been demonstrated that renal denervation partially prevents or alleviates hypertension in one kidney, one clip (1K/1C) rats. Last year it was reported that cutting the $T_8$-$L_2$ dorsal root nerves (the nerves through which the renal afferents pass) similarily reduces the hypertensive effect in this model. In the present study, two further controls experiments have been carried out, to clarify the interpretation of that finding. The first experiment assessed the possibility that lesion of the $T_8$-$L_2$ dorsal root nerves ipsilateral to the remaining kidney, would reduce blood pressure in non clipped, normotensive rats. Four groups of rats were employed: sham (1K/1C), denervated (1K/1C in which the $T_8$-$L_2$ dorsal root nerves were transected ipsilateral to the remaining kidney); control (1K), and denervated control (1K, with ipsilateral lesion as above). The results demonstrated that the lesion had no effect on blood pressure. Final systolic blood pressures five weeks after clipping were: Sham = 179±5mm Hg, denervated = 148±4mm Hg, denervated control = 131±3mmHg and control = 127±5mm Hg.

The second experiment examined the possible antihypertensive effect of the loss of non-renal afferents that are cut along with the renal afferents in the $T_8$-$L_2$ dorsal root nerves. Four groups of rats were included: Sham, denervated, contralateral denervated (1K/1C with lesion of the $T_8$-$L_2$ dorsal root nerves contralateral to the remaining kidney) and control. The results indicate that the non-renal afferents in the $T_8$-$L_2$ dorsal root nerves do not play an important role in the antihypertensive effect. From 4-8 1/2 weeks after clipping, systolic blood pressures of the contralateral denervated and sham groups were indistinguishable from each other and each was significantly higher than the denervated group. Mean systolic blood pressures at week 8 were: Sham = 209±4mm Hg; contralateral denervated = 202±4mm Hg, denervated = 171±3mm Hg, control = 143±5mm Hg. It should be noted that the renal afferent lesion eliminates approximately 50% of the hypertensive effect in the 1K/1C rats. These results demonstrate that the antihypertensive effect of $T_8$-$L_2$ dorsal rhiztomy is not dependent upon the lesion of non-renal afferents. Further, it should be noted that 1) direct arterial blood pressures taken at week 8 1/2 were in close agreement with the indirect tail cuff measures taken at week 8 in experiment 2, 2) there were no differences in mean body weights of the three groups of clipped animals in experiment 2, 3) the dorsal rhizotomies in the denervated group, do not alter sodium handling by the kidney, and therefore the antihypertensive effect does not appear to be dependent upon a renal efferent nerve alterations.

294.18 GUANABENZ-PROMOTED ANTIHYPERTENSION IN THE RAT: ROLE OF NUCLEUS RETICULARIS GIGANTOCELLULARIS AND VAGAL MECHANISM. H.C. Lim* and Samuel H.H. Chan. National University of Singapore, Faculty of Medicine, Kent Ridge, Singapore, and Washington State University, College of Veterinary Medicine, Pullman, WA 99164-6520.

The antihypertensive agents guanabenz and clonidine may share common mechanisms in their cardiovascular effects because they have similar pharmacologic profiles and exhibit mutual interactive actions on the circulatory system (Ong and Chan, Exp. Neurol. 86: 105, 1984). Although guanabenz-promoted cardiovascular suppression has been attributed to its activation of $\alpha$-adrenoceptors located at the bulbar level, a gap still exists in our knowledge as to the specific brainstem locus(i) involved. Since the nucleus reticularis gigantocellularis (NRGC) and vagal mechanism were shown to be critically engaged in clonidine-induced hypotension and bradycardia, the present study was undertaken to evaluate the role of these two mechanisms in similar guanabenz actions, using pentobarbital anesthetized male Sprague-Dawley rats.

Intravenous injection of guanabenz (10 µg/kg) produced an initial, though transient hypertension, accompanied by an increase in cardiac contractility. These were followed by a significant and sustained hypotension, together with a decrease in force and rate of heart contraction. The same administration, delivered to rats that received bilateral electrolytic lesions (1.5 mA, 10 s, d.c.) of the NRGC, however, resulted in only the transient vasopression. The subsequent reduction in arterial pressure, cardiac contractility and heart rate, on the other hand, was sufficiently, though not completely, retarded.

Microinjection of guanabenz directly into the NRGC, at an ineffective systemic concentration (500 ng in 500 nl over 4-5 min), produced significant and prolonged hypotension as well as negative inotropic and chronotropic effects, without the initial hypertension. There was also topographic sensitivity to guanabenz in this reticular nucleus, with the lateral portion of NRGC being significantly less effective in eliciting cardiovascular suppression than its ventro-medial counterpart.

Bilateral cervical vagotomy, performed after significant reduction in arterial pressure, cardiac contraction force and rate was effected following microinjection of guanabenz (500 ng) into the NRGC, appreciably reversed, but did not abolish, the established circulatory depression.

It is concluded that the NRGC is at least one of the central sites involved in guanabenz-promoted cardiovascular suppression. This antihypertensive agent may activate neurons in the ventromedial NRGC, which in turn produces hypotension as well as negative inotropic and chronotropic effects via the vagal outflows to the circulatory effectors.

294.19 EVIDENCE FOR A ROLE OF CARDIAC ATRIOPEPTINS IN REDUCED RENAL MASS PERINEPHRITIC HYPERTENSION IN THE RAT. R.M. Chinn and D.K. Hartle. Dept. of Pharmacology, Emory Univ. Sch. of Med., Atlanta, GA 30322.

Perinephritic hypertension was produced in 200-300 g male Sprague-Dawley rats by loosely wrapping the left kidney with plastic and removing the contralateral kidney. Control rats were subjected to right nephrectomy only. Blood pressure was significantly elevated in the perinephritic rats within 3 weeks after surgery. Rats were anesthetized with pentobarbital and diuresis was induced by continuous infusion of an electrolyte solution (Plasma-Lyte, pH 7.4) at a rate of 0.181 ml/min.Urine was collected in consecutive 10 min. periods. The diuretic, natriuretic, blood pressure and heart rate responses of the perinephritic and control rats were recorded before and after administration of a pharmacological dose of Atriopeptin III (AP III) (10 µg/0.1 ml of infusate, via the jugular vein). AP III was obtained from Peninsula Labs. The responsiveness of the rats to Lasix and Diuril was also tested. Perinephritic rats were unresponsive to the diuretic and natriuretic effects of AP III, but responded well to Lasix and Diuril. These diuretic and natriuretic deficits to AP III were not observed in two other hypertensive reduced renal mass models, Grollman wrap and DOCA/Salt. Blood pressure was lowered significantly more in perinephritic rats than in the controls. In addition, the depressor event was markedly prolonged in the perinephritic rats. AP III increased the rate of diuresis 800% and the rate of natriuresis 1300% in the one-kidney control group. Studies in conscious rats indicated that hypertension in the perinephritic rats is supported by a combination of endocrine (angiotensin and vasopressin) and neural factors. Bioassay of the heart atrial content of diuretic and natriuretic activity indicated that perinephritic rats appeared to have a normal heart content. In summary, anesthetized perinephritic rats appear to be hyperresponsive to the depressor effects of AP III but are unresponsive to the natriuretic and diuretic activities of the peptide. The inability of the perinephritic kidney to respond to AP III is probably due to altered hemodynamic mechanisms associated with AP III action since the kidney was capable of brisk diuresis/natriuresis in response to tubular diuretics (Lasix, Diuril). Because AP III has been shown to lower blood pressure by decreasing cardiac output, the sensitivity of the perinephritic rats to the depressor effects of the peptide indicates that increased cardiac output may be contributing significantly to the support of hypertension in these rats. The impaired perinephritic renal response to AP III may contribute to the process of hypertension, by enhancing sodium retention and increased extracellular fluid load in this reduced renal mass model. Supported in part by AHA, Georgia Affiliate, and PHS#T35-HL30706-06A1.

294.20 SEROTONIN-GABA INTERACTIONS IN DOCA/NaCl HYPERTENSION R. Dawson, S. Nagahama and S. Oparil. CVRTC, Univ. Alabama in Birmingham,Birmingham, AL 35294

The sympathetic nervous system (SNS) is thought to be involved in the genesis and/or maintenance of deoxycorticosterone (DOCA)/NaCl hypertension. The neural mechanisms that augment sympathetic tone in DOCA/NaCl hypertension are poorly understood. Electrical stimulation of the midbrain raphe nuclei or activation of postsynaptic serotonin (5-HT) receptors in the CNS results in increased sympathetic outflow. Conversely, stimulation of the CNS GABA receptors reduces the activity of the SNS. The present study examined the role of central serotonergic mechanisms in DOCA/NaCl hypertension and the effects of a centrally administered GABA agonist on 5-HT metabolism.

Sprague-Dawley rats that had received 4 weeks of DOCA/NaCl treatment were killed by decapitation and monoamine and metabolite levels were determined in brain regions by high performance liquid chromatography and electrochemical detection. DOCA/NaCl hypertensive and control rats were administered 1 µg of muscimol or saline vehicle intracerebroventricularly (icv) and killed 15 or 60 min. later (N=10-15/group).

DOCA/NaCl treated rats had significantly higher basal blood pressure than controls and the dose of muscimol used had previously been shown to produce a significantly greater depressor response in DOCA/NaCl rats than controls. Fifteen min. after icv saline infusion there were no significant differences in 5-hydroxyindoleacetic acid (5-HIAA) levels between DOCA/NaCl rats and controls in any brain region examined. Muscimol (15 min.) produced significant ($p<0.05$) elevations in 5-HIAA and 5-HT levels in the hypothalamus, medulla and pons of DOCA/NaCl rats, however, 5-HIAA and 5-HT levels were not affected in controls. Sixty min. after icv saline infusion, 5-HIAA levels were significantly ($p<0.05$) greater in DOCA/NaCl rats than controls in the spinal cord, medulla, pons, midbrain and hypothalmus. 5-HIAA levels were significantly ($p<0.05$) elevated in control rats 60 min. after muscimol infusion in all brain regions examined. DOCA/NaCl rats exhibited only a slight increase in 5-HIAA levels 60 min. after muscimol.

The results suggest that DOCA/NaCl treatment alters 5-HT metabolism in the CNS and that activation of CNS GABA receptors exacerbates the abnormality in 5-HT metabolism present in DOCA/NaCl hypertensive rats. Thus alterations in CNS GABA and/or 5-HT neurotranmission may contribute to the increased sympathetic tone present in DOCA/NaCl hypertensive rats.

294.21 GABA STRUCTURAL ANALOGUES: RECEPTOR BINDING AND CARDIOVASCULAR EFFECTS IN THE CAT. G. K. Matheson, E. Freed* and G. Tunnicliff*. Indiana University School of Medicine, Evansville, IN 47714.

Several classes of drugs are available for the treatment of hypertension. None, however, is free of side effects, some of which can be serious. A potential new class of drugs to treat high blood pressure is one that enhances gamma-aminobutyric acid(GABA) function in the central nervous system. Evidence existsthat activation of certain central GABA receptors can leadtoa fall in arterial pressure and a decrease in heart rate. On the other hand, antagonism of these same receptors can give rise to an increase in both blood pressure and heart rate.

For this study we measured the ability of over one hundred previously untested structural analogues of GABA to inhibit $GABA_A$ receptor binding in cortical membranes. Compounds found to have an effect on binding were morpholinopropanesulfonic acid (MOPS; $IC_{50}$ = 1.6 μM), 5-phenylpyrrolepropionic acid (5PPP; $IC_{50}$ = 15 μM), aminoethanethiosulfonic acid (AETS, $IC_{50}$ = 22 μM), DL-3-amino-3-phenylpropionic acid (3APP; $IC_{50}$ = 34 μM), m-aminobenzoic acid (MABA; $IC_{50}$ = 58 μM), and urocanic acid (UCA; $IC_{50}$ = 353 μM). To date four of these compounds, along with GABA, 3-aminopropanesulfonic acid and imidazoleacetic acid, have been infused at various concentrations into the fourth ventricle of the alpha-chloralose anesthetized cat, and blood pressure and heart rate were monitored. For blood pressure the $ED_{50}$ values ($1 \times 10^{-8}$ M/Kg) were calculated and are as follows: GABA = 1.95; 3-APS = 1.93; 3APP = 2.79; MABA = 3.36; 5PPP = 4.06; and MOPS = 4.39. We have demonstrated that GABA structural analogues that are capable of inhibiting $GABA_A$ receptor binding are also able to modulate blood pressure in the intact cat and that the two sets of data exhibit a positive correlation.

294.22 POSSIBLE INTERACTION OF SYMPATHETIC REACTIVITY, PAIN AND EMOTIONAL DISTRESS IN ACUTE POSTOPERATIVE HYPERTENSION. E.A. Anderson, Anesthesia Dept., Univ. of Iowa, Iowa City, IA 52242.

Coronary artery bypass graft (CABG) surgery patients have a uniquely high incidence ( 50%) of sympathetically mediated, acute postoperative hypertension (APH) occurring as patients awaken 1-4 hr. after surgery. Prior research suggests that APH patients may be sympathetically hyperreactive. This hypothesis was tested by comparing BP and HR responses of APH and non-APH patients to the intraoperative stimulus of tracheal intubation (TI; which elicits a sympathetic response). It has also been noted that emotional distress may contribute to sympathetic activity after surgery. This hypothesis was tested by comparing the incidence of APH in patients receiving extensive psychological preparation for surgery with those receiving routine preparation. Finally, there is evidence that the cardiovascular system and pain regulatory systems interact: pain increases BP and elevated BP can reduce perceptions of pain. It is possible that APH patients are more sensitive to pain and that increased BP after surgery may functionally reduce pain. The hypothesis of greater pain sensitivity was tested by comparing APH and non-APH patients' requests for analgesics during recovery (postoperative days 2-7; after BP has returned to normal).

60 male CABG patients were randomly assigned to one of two groups receiving extensive psychological preparation for surgery (which successfully reduced pre and postoperative anxiety) or to a control group receiving routine preparation (a short visit by a nurse; N=20/gp). Operative anesthetic was fentanyl-$O_2$. BP and HR were recorded immediately prior to TI and 1 min post TI. Postoperatively, diagnosis of APH was made if a patient was treated with a vasodilator for SBP$\geq$150 mm Hg.

Patients later developing APH (N = 32), had significantly greater SBP (16.5±2.2 vs 6.7±1.6 mm Hg; $p<.001$) and HR 4.1±1.8 vs 0.7±1.6 bpm; $p<.01$) responses to TI than non-APH patients. Second, the incidence of APH was significantly less in the two groups of patients (N=40) extensively prepared for surgery compared to the 20 patients receiving routine preparation (42.5% vs 75%; $p<.02$). Psychological preparation for surgery did not lower BP and HR responses to TI, suggesting that psychological stress and sympathetic reactivity are independent factors interacting in APH. Finally, APH patients requested more analgesics during recovery than non-APH patients (72.4±5.5 vs 54.9±4.0 mg morphine; $p<.04$), a difference not attributable to staff policy.

The results suggest that APH patients are sympathetically more reactive and may have lower pain thresholds than non-APH patients. Finally, reducing patients' anxiety by psychological preparation for surgery can reduce the incidence of APH.

294.23 FUNCTIONAL CHARACTERISTICS OF RAT AORTAE FOLLOWING PARATHYROID-THYROIDECTOMY AND DOCA HYPERTENSION. G.L. Pullen*, T.R. Hansen*, S.P. Singh*, S. Goldman* and J. Cheng*. (SPON: P.C. Tang). Endocrine Research Lab., VA Medical Center, and Univ.Health Sci./ Chicago Med. School, North Chicago, IL 60064.

The purpose of this study was to determine what functional changes occur in the smooth muscle of the rat aorta following 3 different time intervals between parathyroid-thyroidectomy (PXTX) and desoxycorticosterone acetate (DOCA) induced hypertension. Male PXTX Sprague-Dawley rats were purchased from Johnson Labs (Bridgeview, IL) and were subjected to the following protocol:

1. PXTX followed by DOCA treatment after 5 days.
2. PXTX followed by DOCA after 9 days, followed by DL-Thyroxine ($T_4$) supplement after an additional 42 days.
3. PXTX followed by DOCA after 30 days, followed by $T_4$ supplement after an additional 21 days.

Blood pressure in the rats delayed 5 days exhibited the normal increase observed following DOCA treatment without requiring $T_4$ supplement. On the other hand, with a 9 or 30 day delay between PXTX and DOCA, pressure did not increase above normal levels until $T_4$ was administered (10 μg, 3X a week). Following $T_4$ supplementation, the 9 day rats showed a rapid rise in blood pressure (within 48 hrs.) to hypertensive levels (above 150 mmHg) while the 30 day animals showed a gradual rise to hypertensive levels following 4 to 5 weeks of $T_4$ treatment.

The functional characteristics of aortic strips from these groups were studied in a muscle bath. Rats delayed 9 and 30 days between PXTX and DOCA were each split into 2 groups: with or without $T_4$ supplementation. Considering the aortic strip response to norepinephrine in rats with a 30 day delay, those rats which received $T_4$ supplements demonstrated significantly less maximum active stress than those which did not receive $T_4$ ($2.23 \pm 0.61$ vs $3.67 \pm 0.78$ $N/M^2 \times 10^4$). In rats delayed only 9 days, the difference between $T_4$ supplemented and non-supplemented was not statistically significant although the same tendency was noted ($2.65 \pm 0.51$ vs $3.27 \pm 1.34$ $N/M^2 \times 10^4$). There was no significant difference in aortic response between these same groups when barium was used as the agonist. These results confirm earlier findings that $T_4$ deficiency prevents the development of DOCA hypertension while replenishing $T_4$ allows blood pressure to increase (Fed. Proc. 43:443, 1984). In addition, current results are suggestive of an effect of $T_4$ on vascular smooth muscle, perhaps receptor mediated, which is produced either directly on the aorta or indirectly through central mechanisms which increase blood pressure.

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294.24 VENTRICULAR DILATION IN THE SPONTANEOUSLY HYPERTENSIVE RAT (SHR). S. Ritter and T.T. Dinh*. College of Veterinary Medicine. Washington State University. Pullman, WA 99164

In the course of our studies of the SHR we observed that the cerebral ventricles of these rats appeared to be dilated in comparison to age-matched Wistar Kyoto (WKY) and Sprague Dawley (SD) rats. Because ventricular pathology could have important implications for the pathophysiology of hypertension, we measured ventricular area in 14-wk-old male SHR and WKY rats (n = 7 per group) obtained from Taconic Farms. Rats were given a lethal dose of pentobarbital and perfused through the heart with a 4% paraformaldehyde solution from a reservoir of fixed height, elevated so that gravity flow would approximate normal arterial pressure. Ventricular area was measured from mounted brain sections using computer assisted image analysis. The ventricular space was measured at three levels: at the level of the anterior commissure, at the level of the subfornical organ and at the level of the posterior commissure. Sections from SHRs and WKYs were chosen carefully so that equivalent levels were analyzed for each group. All ventricular space in a given section was summed. Our results show that the ventricular space of SHRs is greatly expanded at all three levels analyzed compared to WKYs. At the level of the anterior commissure, ventricular space occupied 5.09 ± 0.56 sq mm in WKYs and 12.10 ± 0.86 sq mm in SHRs. At the level of the subfornical organ and posterior commissure, ventricular space occupied 8.82 ± 0.74 and 14.69 ± 0.92 sq mm, respectively in WKYs and 15.81 ± 1.10 and 23.33 ± 2.36 sq mm, respectively in SHRs. Thus, at these 3 levels, ventricular space of SHRs was 238%, 179% and 159% of the ventricular space in the equivalent sections from WKYs. Tissue surrounding the ventricles in the SHRs showed a parallel attrition in some loci, such as the temporal cortex.

In order to determine whether the apparent hydrocephalus in SHRs is related to their hypertension or is the result of an unrelated genetic defect, we analyzed ventricular size in SHR, WKY and SD rats of different ages: 1 day, 13 days, and 4, 8, 12, and 16 weeks of age. WKYs and SDs did not differ at any age. In SHRs, ventricular dilation was not apparent at 1 day, 13 days or 4 wks of age, when compared to controls. However, 1 out of 3 SHRs thus far examined at 8 wks was hydrocephalic and all rats 12 wks and over were hydrocephalic. The ventricular dilation appeared to be progressive between 12 and 16 wks of age. Thus, the ventricular dilation appears to develop over time and therefore may be related to the hypertensive condition. Experiments are underway to determine whether this condition exists in other forms of experimental hypertension or in other hypertensive rat strains.

294.25 EFFECTS OF SODIUM LOADING UPON CARDIOVASCULAR REGULATORY MECHANISMS. A.F. Tramposch*, K.B. Brosnihan and C.M. Ferrario. Research Division, Cleveland Clinic Foundation, Cleveland, OH 44106.

Previous experiments have shown that in dogs a positive sodium balance causes a marked potentiation of the cardiovascular pressor response due to acute carotid sinus hypotension (Tramposch et al.: The Physiologist 27: 4, 1984). Since the disturbance in baroreceptor function was observed after 14 days of exposure to high sodium ($Na^+$) intake it is possible that a longitudinal characterization of the hemodynamic and neurohormonal events associated with an increase in the concentration of $Na^+$ in the extracellular fluid may provide an insight into the mechanism(s) responsible for the alteration in the baroreflex control of the circulation. Accordingly, 6 male mongrel dogs were instrumented with arterial and venous catheters and an electromagnetic flow probe around the ascending aorta. Following convalescence, their mean arterial pressure (MAP), heart rate (HR), stroke volume (SV), cardiac output (CO) and total peripheral resistance (TPR) were recorded for 5 days before and 14 days during a continuous intravenous (IV) infusion of a 3 molar (M) sodium chloride (NaCl) solution (sodium-loaded (SL) group) or a 0.15 M solution of NaCl (normal (N) sham-group); in all cases the rate of infusion was 4 ml/hr. At frequent intervals throughout the experiment samples of arterial blood were collected for the assay of plasma renin activity (PRA), angiotensin II immunoreactivity (Ang II-ir) and plasma arginine vasopressin (AVP). Plasma norepinephrine (NE), epinephrine (EPI) and $Na^+$ concentrations were also determined. A 14 day IV infusion of 3M NaCl did not produce any consistent changes in either MAP (90 ± 1 in SL vs 93 ± 1 mmHg in N dogs), HR (82 ± 3 in SL vs 85 ± 3 beats/min in N group), SV (37 ± 1 in SL vs 34 ± 1 ml in N group), CO (2931 ± 97 in SL group vs 2848 ± 53 ml/min in N group) and TPR (3.4 ± 0.3 in SL group vs 3.3 ± 1 U in N group of dogs). On the other hand, serum $Na^+$ was increased in the SL group compared to the N group (152.3 ± 0.9 vs 146.5 ± 0.4 mEq/l respectively, $p < 0.05$). The hypernatremia was associated with a 52% decrease in PRA whereas plasma Ang II-ir fell to 0.4 ± 0.1 compared to 12.6 ± 0.1 pg/ml in the N group ($p < 0.05$). Although plasma volume did not change, there was a sustained elevation in plasma AVP concentrations (8 ± 0.4 in SL group vs 2 ± 0.3 pg/ml in N group, $p < 0.05$). Measurements of plasma NE and EPI concentrations revealed an increase in the levels of both NE and EPI in the SL group compared to the N group (406 ± 51 vs 279 ± 30 pg/ml and 239 ± 23 vs 172 ± 24 pg/ml respectively).

These data indicate that chronic $Na^+$ loading is associated with "an adaptive" response of the mechanism involved in the maintenance of base line MAP throughout the infusion period since sustained elevations of plasma markers of two recognized pressor systems (sympathetic and vasopressin) had no hemodynamic effect.

294.PO CATECHOLAMINE - CONTAINING INTRARENAL NERVE ENLARGEMENTS. D.S. Knight* (Spon: K.W. Barron). Dept. of Anatomy, L.S.U. Sch. of Med., Shreveport, LA 71130.

The electron microscope was used to study catecholamine-containing nerve enlargements that are associated with the renal arterial trees of male Wistar and Sprague-Dawley rats. Animals were perfused with 500 milliliters of 4% paraformaldehyde and 1% glutaraldehyde in 0.1 M Millonig's phosphate buffer. The Kidneys were then immersed in this fixative for three hours. Two hundred micrometer thick sections of the kidneys were examined with a fluorescence microscope, and specifically fluorescent structures were excised, embedded in Maraglas, thin sectioned and examined. Perivascular neural structures that contain clusters of vesicles and catecholamine have an identical ultrastructure in male Wistar and Sprague-Dawley rat kidneys. Each such structure consists of a small predominantly unmyelinated nerve bundle with two enlarged segments. Between the two enlargements, the nerve forms a thin band that contacts the outer medial surface of an arteriole. The two enlargements differ ultrastructurally. One enlargement consists of Schwann cells and axonal swellings each of which contains a central large cluster or several smaller clusters of agranular and dense-core vesicles 50 to 70 nanometers in diameter. Axonal swellings that comprise the other type of nerve enlargement are packed with microtubules, bundles of neurofilaments and small mitochondria. These swellings are more elongate than those containing vesicle clusters and are only partially covered by Schwann cell processes. There are myelinated axons of predominantly microtubular or neurofilamentous content in the nerve enlargements. Some axons lose the myelin sheath and may terminate in the nerve enlargements composed of vesicle-filled axonal swellings.

## PHARMACOLOGY OF SYNAPTIC TRANSMISSION III

295.1 INHIBITION BY NIMODIPINE OF BAY K 8644 AND KCl-STIMULATED CALCIUM ENTRY INTO AND ENDOGENOUS DOPAMINE RELEASE FROM STRIATAL SYNAPTOSOMES. John J. Woodward* and Steven W. Leslie. Division of Pharmacology, College of Pharmacy, Univ. Texas at Austin, Austin, TX 78712.

Recent studies have demonstrated the existence of high affinity $^3$H-nimodipine binding sites in brain. These studies, reporting $k_d$ values in brain similar to those obtained in cardiovascular tissue, suggest the existence of dihydropyridine-sensitive calcium channels in brain. However, other recent reports have shown that calcium channels in brain are insensitive to blockade by calcium entry blockers. Thus, there is a question as to whether or not dihydropyridine-sensitive calcium channels exist in brain. To pursue this question, the effects of nimodipine on voltage-dependent calcium entry into and endogenous dopamine release from striatal synaptosomes have been studied in response to KCl-stimulation and KCl plus the agonist BAY K 8644. Synaptosomes ($P_2$ pellets) were isolated from striata of male, Sprague-Dawley rats (approximately 300g) and preincubated at 30°C for 14 minutes (Leslie et al. Brain Res. 325, 99, 1985), in the presence or absence of nimodipine, BAY K 8644 or nimodipine plus BAY K 8644. $^{45}Ca^{++}$ entry and endogenous dopamine release under resting conditions (5mM KCl) or in response to depolarization (KCl, 15 or 30mM) were determined over a 3 second time period as previously described (Leslie et al. Brain Res. 325, 99, 1985). Nimodipine, 0.1nM to 10.0μM, did not alter resting calcium accumulation or dopamine release. Nimodipine, 0.1nM - 10.0μM, had no effect on 30mM KCl-stimulated calcium entry or dopamine release except for a slight stimulation of release with 0.1nM nimodipine and a slight reduction in release with 10.0μM nimodipine. These findings agree with previous reports suggesting a lack of effect of calcium entry blockers under these conditions. BAY K 8644, 0.1nM - 100nM, did not alter resting calcium entry or dopamine release but significantly enhanced 30mM KCl-stimulated calcium entry. A similar (but not significant) tendency for increased dopamine release by BAY K 8644 in response to 30mM KCl was observed. The combination of BAY K 8644, 1nM, plus KCl, 15mM, resulted in a significant 26.5 percent increment in calcium entry and 11 percent increment in dopamine release above that observed with 15mM KCl-depolarization alone. The BAY K 8644 increment in calcium entry and dopamine release was inhibited by nimodipine, 10nM. These studies show that the addition of 1mM BAY K 8644 to partially depolarized synaptosomes (15mM KCl) results in an incremental increase in calcium uptake and dopamine release that is sensitive to blockade by the calcium entry blocker nimodipine. The combination of BAY K 8644 and partial KCl-depolarization may unmask dihydropyridine sensitive calcium channels.

295.2 SLOW HYPERPOLARIZING POTENTIALS IN RAT SPINAL DORSAL HORN NEURONS. S. Jeftinija, L. Urbán* and M. Randić. Depts. of Vet. Anat. and Physiol. and Pharmacol., Iowa State Univ., Ames, IA 50011.

High intensity repetitive stimulation of a dorsal root elicits slow hyperpolarizing potentials in a portion of rat spinal dorsal horn neurons studied in vitro (Urbán and Randić, Brain Res., 290:336, 1984). The present experiments were designed to test the hypothesis that slow hyperpolarization is a synaptic event and to obtain information about possible mediators responsible for its generation. Norepinephrine and enkephalins are considered as inhibitory neurotransmitter candidates since they hyperpolarize rat spinal dorsal horn neurons (Murase et al., Brain Res., 234:170, 1982; North and Yoshimura, J. Physiol., 349:43, 1984).

Rats 10-30 days old were used. After lumbosacral laminectomy 300-400 μm thick horizontal or transverse spinal cord slices were made. Intracellular recordings from dorsal horn neurons were performed with micropipettes filled with 3 M K-acetate or 2 M KCl. Tested compounds were dissolved in Ringer solution and applied in known concentrations to the slices by perfusion.

High intensity repetitive stimulation of a dorsal root (10-25 V pulses of 0.2-0.5 ms duration applied at 10-20 Hz for 2-5 s) elicited two types of potential changes in dorsal horn neurons located in laminae I-V. In 17 cells a triphasic potential change was observed; the initial fast excitatory synaptic potentials were followed by a hyperpolarization which was in turn succeeded by a slow depolarization. When recorded at resting membrane potentials of -60 to -75 mV, the mean duration of the hyperpolarization was 24±11 s (mean±S.D.) and the amplitude 8±3 mV. In addition, in substantia gelatinosa cells and some of lamina V cells a biphasic potential change was observed; the initial fast excitatory synaptic potentials were followed by a slow hyperpolarization of 55±24 s with the mean amplitude of 9±6 mV (n=19). A low $Ca^{2+}$/high $Mg^{2+}$ solution or tetrodotoxin ($10^{-6}$M, n=3) reversibly abolished both types of hyperpolarizations. The two hyperpolarizing potentials were associated with a decrease in neuronal input resistance. Bath application of (D-$Ala^2$, $Met^5$)- or (D-$Ala^2$, $Leu^5$)- enkephalinamide (5 x $10^{-6}$M, CRB) and norepinephrine (NE, $10^{-7}$ to $10^{-6}$M, Regis) reversibly hyperpolarized the same neurons having slow hyperpolarizing potentials. Naloxone ($10^{-6}$M, n=7) and the yohimbine ($10^{-7}$ to $10^{-6}$M, n=3) reduced both types of the slow hyperpolarizations. The two hyperpolarizing potentials were enhanced, rather than depressed, by bicuculline ($10^{-5}$M, n=4).

The present findings demonstrate the presence of two types of slow hyperpolarizing potentials in dorsal horn neurons. In addition, the data suggest that NE and/or enkephalins may serve as inhibitory neurotransmitters.

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295.3 EFFECTS OF POLYCLONAL AND MONOCLONAL ANTIBODIES TO SUBSTANCE P ON SLOW EXCITATORY TRANSMISSION IN RAT DORSAL HORN. M. Randić, L. Urban,* P. D. Ryu,* and R. Chin.* Dept. of Vet. Physiol. and Pharmacol., Iowa State Univ., Ames, IA 50011 and Dept. of Anatomy, Univ. Medical School, Debrecen H 4012, Hungary.

We have demonstrated that high intensity repetitive stimulation of a dorsal root elicits a slow depolarization in dorsal horn neurons that may be mediated by a substance P (SP) or a SP-like peptide (Urban and Randić, Brain Research, 290:336, 1984). In an attempt to provide a further support for the neurotransmitter role of SP, effects of bath perfusion of polyclonal and monoclonal antibodies to SP on slow excitatory transmission in rat dorsal horn neurons have been investigated by intracellular recording in the immature rat (13-24 days old) spinal cord slice preparation.

Both polyclonal (diluted to 1:50 or 1:100; n=25) and monoclonal (1:12.5 to 1:50; n=6) SP antisera produced a substantial decrease in the level and duration of the slow depolarization generated in dorsal horn neurons by dorsal root stimulation or exogenous SP application. In those cells where the slow depolarization was accompanied by an increase in fast synaptic activity and firing of action potentials, the SP antisera markedly suppressed the synaptic activity and firing. The effect on the dorsal root-elicited slow depolarization appeared within 10-40 min following the onset of perfusion, and after the removal of SP antisera, the response only partially recovered with a relatively slow time course. The effect of endogenous SP released during dorsal root stimulation appears likely since bath perfusion of a slice with a normal rabbit serum, or affinity chromatography adsorbed SP antiserum, or non-specific IgG, had no similar depressant effect.

The effects of SP antisera, if taken together with other experimental evidence, suggest that SP or a SP-like peptide, is in some way involved in generation of the dorsal root elicited slow depolarizing response. In addition, a novel approach is presented for using polyclonal and monoclonal antibodies to SP as pharmacological antagonists in vitro.

(For generous donation of SP antisera, we are most grateful to Dr. T. M. Jessell, Harvard Medical School. Supported by NIH grant NS 17297, National Science Foundation grant BNS 8418042, and the United States Department of Agriculture.)

295.4 EFFECTS OF 4-AMINOPYRIDINE ON SYNAPTIC CURRENTS IN CA3 HIPPOCAMPAL NEURONS. P.A. Rutecki*, F.J. Lebeda, and D. Johnston. Dept. of Neurol. & Neurosci. Prog., Baylor Col. of Med., Houston, TX 77030.

4-aminopyridine (4-AP) is a potent convulsant that produces synaptically mediated epileptiform activity in the hippocampal slice. The paroxysmal depolarizing shift (PDS) produced by 1-10 μM 4-AP is a complex waveform compared to the PDS produced by GABA antagonists. Several lines of evidence suggest that the convulsant properties of 4-AP are not due to a decrease in GABA-mediated synaptic inhibition. In order to test this hypothesis further, we have begun to study the effects of 4-AP on synaptic responses in CA3 pyramidal neurons under voltage clamp.

Rat hippocampal slices were prepared in a conventional manner. Intracellular recordings were made from CA3 pyramidal neurons using low resistance microelectrodes and a single-electrode current- and voltage-clamp system. Synaptic currents were evoked at 0.2 Hz by stimulating dentate granule cells with a bipolar stimulating electrode.

In control saline, the evoked synaptic currents at potentials near rest consisted of an initial inward current followed by an outward current. Measurements of the currents at different holding potentials were made at 15 ms following the stimulus. Previous studies have shown that the current at this delay from the stimulus results primarily from recurrent and/or feedforward inhibition (Brown and Johnston J. Neurophysiol. 50:487). In 10 neurons the measured synaptic current at this 15 ms time point had a mean conductance of 74.6 ± 7.9 nS and a mean reversal potential of -66.7 ± 1.3 mV. Measurements of the evoked synaptic currents were obtained before and at various times after adding 1-5 μM 4-AP to the bathing solution. In half the neurons studied there was an increase (>30%) in the 15 msec conductance measurement. In the other half there was either no change or a small decrease in conductance. Experiments are being conducted adding picrotoxin to the bath to evaluate the effects of 4-AP on pure excitatory synaptic currents.

These results support the hypothesis that the convulsant properties of 4-AP are not due to disinhibition and may even be accompanied by an increase in inhibition. We suggest that epileptiform activity can be observed in hippocampal slices in the presence of normal (and even increased) synaptic inhibition. (Supported by the Grass Foundation, BRSG-RR05425, USAMRDC DAMD17-82-C-2254, and NIH grant NS11535.)

295.5 L-2-AMINO-4-PHOSPHONOBUTYRATE ACTS PRESYNAPTICALLY TO BLOCK NEUROTRANSMISSION IN THE HIPPOCAMPUS. Alan H. Ganong, Martin N. Perkins*, John A. Flatman*, and Carl W. Cotman. Department of Psychobiology, University of California, Irvine, California 92717.

The majority of excitatory synapses in the mammalian CNS probably use L-glutamate or a closely related amino acid as a transmitter. The L-glutamate analogue L-2-amino-4-phosphonobutyrate (L-AP4) is a potent and selective antagonist at several CNS excitatory pathways, but its mechanism of action is unknown. We have analyzed the effects of L-AP4 and several other synaptic antagonists on evoked and spontaneous EPSPs at the L-AP4—sensitive mossy fiber—CA3 pyramidal cell synapse in guinea pig hippocampal slices.

Intracellularly recorded stimulus-evoked mossy fiber EPSPs were strongly antagonized by 50 μM L-AP4 (89±2% inhibition, n=10), but were not affected by 50 μM D-AP4. The L-AP4 analogue L-serine-O-phosphate (L-SOP) applied at 200 μM also antagonized evoked EPSPs (67±2% inhibition, n=4). The presumed postsynaptic antagonists kynurenate (400 μM) and N-(p-bromobenzoyl)piperazine-2,3-dicarboxylate (200 μM, pBB-PzDA) also blocked evoked mossy fiber EPSPs by 64±5% (n=8) and 68±2% (n=6).

The effects of these synaptic antagonists on spontaneous mossy fiber miniature EPSPs (mEPSPs) fell into two groups. L-AP4 and L-SOP, applied at concentrations that blocked evoked EPSPs, did not affect amplitude distributions of spontaneous mossy fiber mEPSPs. In the presence of 50 μM L-AP4, spontaneous mEPSPs were recorded that ranged in amplitude similar to the amplitudes of events in control medium; the amplitude distributions of spontaneous mEPSPs were not affected by L-AP4 (8 of 9 cells, $p > .2$, Kolmogorov-Smirnov statistic). Spontaneous mossy fiber mEPSPs were also not affected by 200 μM L-SOP in 3 of 4 CA3 pyramidal neurons ($p > .2$). In one cell to which L-SOP was applied, there was an increase in mEPSP amplitude that persisted in the wash.

In contrast to the actions of L-AP4 and L-SOP, kynurenate and pBB-PzDA consistently reduced the amplitude distributions of spontaneous mossy fiber mEPSPs. Kynurenate (400 μM) reduced the amplitude of spontaneous mEPSPs in 4 of 4 neurons ($p < .01$). pBB-PzDA (200 μM) also reduced the amplitude of mossy fiber mEPSPs in 4 of 4 cells ($p < .01$).

The lack of effect of L-AP4 on the amplitude distribution of spontaneous mEPSPs at a concentration that virtually abolishes stimulus-evoked EPSPs, together with previous demonstrations of the weak action of L-AP4 as an antagonist of amino acid-induced excitations, demonstrates that L-AP4 (and L-SOP) act by way of a presynaptic receptor. The ability of kynurenate and pBB-PzDA to antagonize both stimulus-evoked and spontaneous EPSPs indicates that these compounds act at postsynaptic receptors. The ability to selectively manipulate pre- and post-synaptic components of synaptic transmission at the guinea pig mossy fiber and other L-AP4—sensitive synapses should lead to new insights into the mechanisms of excitatory amino acid neurotransmission in the CNS. (Supported by DAMD 17-83-C-3189).

295.6 PEROXIDATIVE DAMAGE IN CA1 HIPPOCAMPAL PYRAMIDAL CELLS OF THE GUINEA PIG. T. C. Pellmar. Physiology Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814-5145.

Free radicals and active oxygen are thought to increase in concentration in the brain following ischemia and reperfusion. The electrophysiological consequences of active oxygen damage were investigated in a hippocampal brain slice preparation. Hydrogen peroxide (0.01%) and $FeSO_4$ (100 uM) ($H_2O_2$/Fe) were added to the Ringers' solution to generate hydroxyl free radicals. Extracellular recordings were obtained from the somatic and dendritic layers of CA1 region. Bipolar electrodes were positioned in the alveus for stimulation of the antidromic pathway and in the stratum radiatum for orthodromic stimulation.

$H_2O_2$/Fe had no effect on the antidromic population spike but greatly reduced the orthodromically elicited population spike. $H_2O_2$/Fe also decreased the dendritic response (pop PSP). Analysis of input-output curves revealed that the decrease in the pop PSP was insufficient to produce the observed change in the population spike. The results suggest that $H_2O_2$/Fe casues both a decrease in synaptic efficacy and impairment of action potential generation. Control experiments showed that 100 uM $FeSO_4$ alone was without effect on the orthodromic response. Peroxide alone (0.01%) produced similar results to $H_2O_2$/Fe but with a slower time course.

Intracellular recordings from CA1 pyramidal cells substantiated the extracellular findings. $H_2O_2$/Fe caused no change in resting membrane potential or membrane resistance. However, the orthodromically elicited EPSP was reduced. In addition, exposure to $H_2O_2$/Fe decreased the number and the firing frequency of action potentials evoked by a depolarizing current step.

These results suggest that free radicals can cause neuronal damage reflected in alteration of electrophysiological properties.

295.7 BIPHASIC EFFECT OF AMINOGLYCOSIDES ON TRANSMITTER RELEASE IN THE POTASSIUM-STIMULATED FROG NEUROMUSCULAR JUNCTION. P.A. Talbot and H.L. Peterson. Department of Pharmacology, Xavier University College of Pharmacy, New Orleans, LA 70125.

Miniature endplate potential (MEPP) frequency is dependent on $Ca^{2+}$ inside the presynaptic nerve terminal. High $K^+$ and other membrane depolarizing factors cause an increase in $Ca^{2+}$ influx into the nerve terminal with a consequent increase in MEPP frequency; an effect which is competitively antagonized by $Mg^{2+}$.

Using intracellular microelectrodes, aminoglycosides were investigated electrophysiologically in tetrodotoxin-blocked sciatic nerve-sartorius muscles. The preparation was superfused continuously with control Ringer's followed by Ringer's containing drug then by control Ringer's again. In 10mM $K^+$-Ringer's (no $Mg^{2+}$) sisomicin sulfate (used at 20ug/ml) consistently caused an immediate increase in MEPP frequency to 123% of control ($p < .01$) which lasted for 1 min and was subsequently followed by a maximal decrease to 24% of control ($p < .02$) at 10 min that remained unchanged in the presence of drug (for 20 min). Similarly, in 10mM $K^+$, amikacin (80ug/ml), tobramycin (80ug/ml), and the sulfate salts of gentamicin (20ug/ml), netilmicin (20ug/ml), kanamycin (80ug/ml), streptomycin (80ug/ml) and neomycin (5ug/ml) produced an initial increase which was followed by a depression to 28% ($p < .05$), 14% ($p < .05$), 24% ($p < .01$), 20% ($p < .05$), 13% ($p < .01$), 40% ($p < .01$) and 20% ($p < .05$) of control, respectively. In 5mM $K^+$-Ringer's, the magnitude of the initial increase caused by sisomicin was not significantly changed; this effect, however, was followed by a decrease that was not significantly different from the control baseline in the presence of drug. When 5mM $Mg^{2+}$ was present in 10mM $K^+$-Ringer's, sisomicin did not cause an effect on MEPP frequency that was significantly different from control.

In conclusion, the aminoglycosides caused a marked depression of $K^+$-stimulated transmitter release consistent with an action of these drugs to block $Ca^{2+}$ influx into the nerve terminal. These drugs also caused, however, an immediate increase in transmitter release that was not dependent on $K^+$ but was susceptible to $Mg^{2+}$ blockade suggesting an additional action of these drugs to depolarize the presynaptic membrane.

(supported by a grant from NIH)

295.8 LINDANE EXERTS BOTH PRE- AND POSTSYNAPTIC ACTIONS AT FROG NEUROMUSCULAR JUNCTION. S. M. Vogel*, R. M. Joy and T. Narahashi, Dept. Pharmacology, Northwestern University School of Medicine, Chicago, IL 60611.

Lindane (gamma-hexachlorocyclohexane) has previously been shown to increase miniature end plate potential (MEPP) frequency and decrease MEPP amplitude at frog neuromuscular junction. In this study we investigated whether the reduction in MEPP amplitude was pre- or postsynaptically mediated.

Cutaneous pectoris muscles were removed from frogs, and conventional microelectrodes filled with 3M KCl were used to record membrane potentials. For iontophoresis a second microelelectrode was filled with 1M ACh which was released by depolarizing pulses of 5-10 msec at a current intensity causing reproducible responses of 3-5 mV.

Perfusion with lindane produced a time and concentration dependent effect on MEPPs. At 50 uM MEPP frequencies were typically increased by 100-500%. MEPP amplitudes were progressively decreased, often becoming undetectable. Effects developed within 10-20 minutes, and full recovery usually occurred within 30-60 minutes.

To test whether the reduction in MEPP amplitude was mediated postsynaptically ACh was applied to the end-plate by iontophoresis. The amplitude of ACh-evoked depolarizations declined with a time course nearly identical to the decline of MEPP amplitude. This indicates that lindane causes a postsynaptic block at the end-plate which can account for the observed decrease in MEPP amplitude. It is unlikely that lindane reduces the quantal size because of the similarity in time course for depression of spontaneously released and iontophoretically applied ACh.

In solutions containing high $Mg^{++}$, evoked EPPs were increased or unchanged in amplitude at the time when MEPP amplitudes were severely depressed. This is interpreted to indicate that lindane also enhances evoked transmitter release. The magnitude of this effect is obscured because of the simultaneous postsynaptic block. These results indicate that lindane produces both presynaptic and postsynaptic actions at frog neuromuscular junction. Presynaptically it increases transmitter release. Postsynaptically it reduces the responsiveness of the end-plate to ACh.

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295.9 ACTIONS OF BARIUM ON FROG SPINAL CORD. J.C. Hackman, G.P. Ryan, G.J. Wohlberg, and R.A. Davidoff. Neurophysiology Laboratory, VA Medical Center, and Depts. of Neurology and Pharmacology, Univ. of Miami School of Medicine, Miami,Fl. 33101.

The divalent cation barium ($Ba^{++}$) has several different effects on excitable tissue. $Ba^{++}$ can replace $Ca^{++}$ to maintain transmitter release at some synaptic junctions, but not at others, and can pass through $Ca^{++}$ channels in non-synaptic membrane. Other data indicate that $Ba^{++}$ interferes with the function of several types of $K^+$ channels. In contrast to $Ca^{++}$ and $Mg^{++}$, $Ba^{++}$ has potent excitatory effects on vertebrate central neurons. The present experiments investigated the effects of the cation on the frog spinal cord.

Sucrose gap recordings were made from the dorsal (DR) and ventral (VR) roots of the isolated,hemisected cord superfused with $HCO_3^-$-buffered Ringer's solution (15°C). $K^+$-sensitive microelectrodes measured changes in extracellular $K^+$ concentrations in the dorsal horn and intermediate gray matter.

Addition of $Ba^{++}$ (25uM-5mM) depolarized both DRs and VRs in a concentration-dependent and reversible manner. Much of this effect appeared to be indirect since $Mg^{++}$ (10mM) and TTX (0.625uM) substantially reduced the $Ba^{++}$-induced depolarization. $Ba^{++}$ (25-500uM) markedly increased the frequency and duration of spontaneous DR and VR potentials. Evoked DR and VR potentials were greatly prolonged in duration and frequently increased in amplitude at the same concentrations. At higher concentrations (1-5mM) both the spontaneous and evoked potentials were reduced in amplitude. However, the duration of the responses remained prolonged.

The increased excitability did not appear to result from increased postsynaptic responses to putative excitatory amino acid transmitters or to $K^+$ since the addition of $Ba^{++}$ reduced motoneuron depolarizations elicited by the application of L-glutamate, L-aspartate (1mM) or $K^+$ (15mM). However,a reduction in pre- and post- synaptic inhibition may be a factor because GABA-induced depolarization of afferent terminals and GABA- and glycine-induced hyperpolarizations of motoneurons were reduced by $Ba^{++}$.

Both the baseline levels and the evoked release of $K^+$ were increased by $Ba^{++}$. At higher concentrations of $Ba^{++}$ (>500uM) the evoked release of $K^+$ was reduced,but still remained above baseline levels.

In sum, low concentrations of $Ba^{++}$ are highly excitatory when applied to the spinal cord. A block of inhibitory amino acid transmitter action and enhanced release of $K^+$ by afferent stimuli appear to contribute to this increased excitation. (Supported by VAMC MRIS #1769 and #3369 and USPHS #NS17577 and #HL07188)

295.10 THE SUCROSE GAP RECORDING TECHNIQUE APPLIED TO THE RAT SUPERIOR CERVICAL GANGLION. D.G. McKenna*, C.A. Briggs, D.M. Barnes and D.A. McAfee. Beckman Research Institute of the City of Hope, Duarte CA 91010.

The sucrose gap technique provides a convenient method for obtaining a direct measure of transmembrane potentials. It can be used to record from a variety of excitable tissues provided that the preparation is large enough to span the gap. We describe here the fabrication of a sucrose gap apparatus for an unusually small preparation - the rat superior cervical ganglion.

Ganglia from young adult rats are fusiform shaped, about 4mm long by 0.8 mm maximum diameter, and have a wet weight of 1 mg. The actual gap is composed of a piece of polyethylene tubing (PE 10) through which isotonic sucrose flows by gravity feed. The internal carotid (postganglionic) nerve is threaded across the stream of sucrose through a 100 um hole pierced transversely in the tubing. The tubing is sealed between two chambers that are electrically isolated from each other and contain Locke's solution flowing at 0.1 ml/min.

The body of the ganglion is in one chamber while the end of the internal carotid nerve is in the other. Calomel electrodes in contact with the two chambers are used to differentially record the voltage across the gap. This voltage will reflect changes in the membrane potentials of the postganglionic axons which span the gap. In addition, potentials will be conducted to the gap from distant somata and dendrites with an estimated electrotonic decrement of 63%/220 um (McAfee, 1982 in Progress in Cholinergic Biology, Hanin and Goldberg eds., Raven press).

The preganglionic nerve is threaded into a third chamber containing Ag bipolar stimulating electrodes. Preganglionic stimulation produces a compound action potential (15 mV) and the following sequence of synaptic potentials: fast-EPSP, slow-IPSP, slow-EPSP, and late slow-EPSP. Brief periods of repetitive preganglionic stimulation produce both posttetanic and long-term potentiation. Increases in extracellular $K^+$ on the ganglion side of the gap depolarize as an exponential function of concentration (12 mV/decade). Nicotinic cholinergic agonists added to the Locke's solution cause depolarization (1 mV at 10 uM). This preparation is especially suitable for the quantitative study of receptor pharmacology. Current studies include receptor mediated activation of cyclic nucleotide and phosphatidylinositol second messenger mechanisms.

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295.11 MEMBRANE AND SYNAPTIC PROPERTIES OF PROOPIOMELANOCORTIN-CONTAINING CELLS. Q.J. Pittman and B.A. MacVicar, Dept. of Medical Physiology The University of Calgary, Calgary, Alberta T2N 4N1, Canada.

The rat pars intermedia (PI) consists wholely of cells of the proopiomelanocortin family whose major secretory products include α-melanocyte stimulating hormone and endorphins. Hormone output from these cells is thought to be inhibited by GABAergic and dopaminergic inputs and stimulated by β-adrenergic agents. We have carried out intracellular recordings in isolated rat PI to examine the membrane properties of these cells and to characterize their synaptic inputs. Pituitaries were placed in a warmed (33° C) recording chamber and superfused with artificial CSF. Afferent fibres were activated with a bipolar electrode placed on the stalk and PI cells were impaled with microelectrodes filled with 2 M K acetate. Some cells were also filled with Lucifer yellow and subsequently these small (10 x 15 μm) rectangular cells were localized to the PI.

Pi cells (N=50) have low resting potentials (-30 to -65 mV) and high input resistance (200-1000 mΩ). Depolarizing current elicited over-shooting action potentials. This was followed by a pronounced after-hyperpolarization which displayed increased conductance and reversal at approximately -80 mV. Action potentials were blocked by TTX (1 μm). In high calcium (10 mM) or barium (10 mM) a calcium spike could be evoked which could be blocked with cadmium (1mM). Stalk stimulation evoked an IPSP which reversed at -60 mV or following intracellular chloride injection (KCl electrode). The IPSP was associated with increased conductance, was of variable duration (0.2-2 s) and decremented with repeated stimulation. Superfusion with $10^{-5}$ M bicuculline, a GABA antagonist, blocked this response. The GABA IPSP was often followed by a slow hyperpolarization which could last up to 12 s. It was associated with a slight (12%) decrease in conductance, was enhanced when the cells were hyperpolarized with negative current and was not readily reversible. This response summated upon repetitive stimulation. It was consistently blocked with the dopamine antagonists, chlorpromazine (50 μm) or domperidone (1 μm).

These results indicate that PI cells are under an interesting form of control wherein membrane potential can be altered for short periods of time by GABAergic inputs and over a much longer time course via a dopaminergic input. The monosynaptic nature of these inputs provides an ideal opportunity for further characterization of actions of identified synaptic transmitters.

(Supported by AHFMR and MRC).

295.12 Ia AFFERENT EXCITATION OF MOTONEURONS IN THE NEWBORN RAT SPINAL CORD IS SELECTIVELY ANTAGONIZED BY KYNURENATE. C.E. Jahr and K. Yoshioka*. Dept. Neurobiol., Harvard Med. Sch.,Boston, MA 02115.

Intracellular recordings from motoneurons of in vitro preparations of newborn rat spinal cord were used to study the sensitivity of the Ia excitatory postsynaptic potential (epsp) to antagonists of excitatory amino acids in order to test whether group Ia primary afferents release L-glutamate, or a similar compound, as a neurotransmitter. The Ia epsp was isolated from afferent evoked polysynaptic input to motoneurons by low intensity stimulation of individual muscle nerves and by the addition of high concentrations of divalent cations to the superfusate which suppressed polysynaptic circuits. The pattern of convergence of group Ia afferents from homonymous, heteronymous and antagonist muscle nerves onto motoneurons in the newborn rat was similar to that reported in the adult cat spinal cord. Homonymous muscle nerve stimulation evoked the largest amplitude Ia epsp while heteronymous muscle nerve stimulation elicited smaller epsps or had no effect. Stimulation of antagonist muscle nerves resulted in inhibitory postsynaptic potentials (ipsps). Superfusion of the specific N-methyl-D-aspartate (NMDA) receptor antagonist, 2-amino-phosphonovalerate, did not inhibit the Ia epsp but did suppress later, polysynaptic components of the dorsal root evoked response. Kynurenate was a potent inhibitor of the Ia epsp. The site of action of kynurenate was examined by observing its effect on synaptic depression and was found to be consistent with a postsynaptic mechanism. Kynurenate selectively blocked the depolarization of motoneurons elicited by L-glutamate and had no effect on the recurrent ipsp evoked by ventral root stimulation with its effect on the Ia epsp. The recurrent ipsp was antagonized by strychnine and dihydro-beta-erythroidine while kynurenate, at a concentration which greatly reduced the Ia epsp, had no effect. These results suggest that stimulation of group Ia primary afferents evoked the release of L-glutamate, or a similar compound, which activated non-NMDA excitatory amino acid receptors on motoneurons which, in turn, mediated the Ia epsp.

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## SENSORY SYSTEMS: SUBCORTICAL VISUAL PATHWAYS III

296.1 LONG AND SHORT TERM EFFECTS OF AN AUDITORY STIMULUS ON VISUAL RESPONSES OF THE SUPERIOR COLLICULUS. S. Molotchnikoff, E. Sicard*, C. Casanova* and P. Lachapelle*. Dépt. de Biologie, Université de Montréal, Qué., Canada, H3C 3J7.

The convergence of visual, auditory and somatic inputs to the superior colliculus represents one of the most interesting aspects of collicular physiology. Evidence has been presented that there are interactions between these three modalities, but the characteristics of these interactions are far from being elucidated. Hence, the scope of the present investigations is to shed more light on the influences of auditory stimuli on light responses.

In anesthetized and paralyzed rabbits single units recordings were taken from superficial layers of the superior colliculus in a standard manner. Each neuron was stimulated with a circumscribed dark or light bar sweeping across its receptive field in the horizontal or vertical direction. An auditory pure tone (duration: 300 ms, 10 $KH_Z$, was added at various delays prior to the light-evoked discharges. One important methodological aspect must be underlined. Since the collicular cells tend to habituate to repetitive stimulus applications, very low frequencies were applied (up to 1 stimulus per 20 sec), when necessary. In addition the sequence between control and test trials was run randomly in blocks of 4 consecutive presentations. Results (N = 199) revealed that the auditory pulses are capable of increasing (N = 50) and decreasing (N = 53) light responses. Two patterns of interactions were noticed: a) a short term effect whose peak time is 50 to 100 ms, and b) a long term effect which may last several seconds. Computations to establish a correlation between the receptive field coordinates of the collicular cell and the magnitude of the influence of the auditory pulse revealed a lack of tight relations between these two variables. In addition, the distance separating the speaker and the collicular receptive field is not related to the amplitude of the alterations of the light responses. These results suggest that the collicular cells work according to a "functional gradient model" (McIlwain, J. Neurophy. 1982: 47) rather than a more rigid point-by-point activation which assumes fixed topographic representations. Finally, a survey of data obtained so far suggests that units influenced by auditory pulses are grouped together because once a neuron is isolated neighbouring cells had a high probability of yielding similar interactions. These latter results further suggest a modular organization of the collicular network in rabbits.

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296.2 INFLUENCE OF THE CRYOBLOCKADE OF THE VISUAL CORTEX UPON LIGHT RESPONSES OF SUPERIOR COLLICULUS CELLS IN RABBITS. L. Lamothe*, S. Molotchnikoff, A. Cérat* and E. Sicard*. (Spon: F. Lepore) Dept. Biologie, Université de Montréal, Montréal, Qué. Canada, H3C 3J7.

It has been demonstrated in cats that collicular responsiveness to visual stimuli depends upon the cortical input. Particularly, movement sensitivity and directional selectivity are depressed with the interruption of cortico-collicular impulses. One of the peculiarities of the rabbit's visual system is that the movement sensitivity is already present at the retinal level. It is therefore justified to study the cortico-collicular influences in this species.

In anesthetized paralyzed rabbits single units recordings are carried out with a NaCl-filled glass micropipette. Each cell was tested for movement and directional sensitivity with appropriate images (bars, slats, etc...) sweeping across its receptive field. The cellular activity was obtained in PSTH form prior to, during and after cortical blockade. Field potentials recorded from the cortex permitted us to assess the cortical disfunction. Of 48 fully tested cells a fairly large proportion (85%, N = 41) were influenced by cortical depression. Thirty-one of these affected cells exhibited a robust decline in their responses. In some cases cells' responses to light were completely eliminated when cortical activity was also abolished. Only a small proportion (10%) of collicular cells reacted with an increase of their excitation concurrent to cortical blockade. Although in most instances, the responses to various trigger features (on-off stimuli, direction of the moving images) declined with the same magnitude, in a few cells the discharge to one particular trigger feature was more affected than it was to others. Finally, a trend seemed to emerge: cells which reacted by a facilitation to cortical blockade appeared to be located more dorsally than units with a depressing influence. These preliminary results indicate that the cortico-collicular influence is relatively homogeneous but in a few neurons the cortical impact exerts a more subtle and specific role. These findings stand in sharp contrast with cortico-geniculate influences. Indeed corticofugal axons which contact the lateral geniculate cells have a very specific action since they influence the center-surround equilibrium in concentric units, to the detriment of the surround in most cases. Thus, the global modulations of the collicular responsiveness by the cortex may be associated with the role attributed to the superior colliculus in alerting and preparing the oculomotor neuronal network to readjust the gaze of the animal and unlock (or lock) the fixation of the eyes.

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296.3 IMMUNOCYTOCHEMICAL LOCALIZATION OF GAMMA-AMINOBUTYRIC ACID (GABA) IN THE CAT SUPERIOR COLLICULUS. R.R. Mize and L.H. Horner*, Div. of Neuroscience, Dept. of Anatomy, University of Tennessee Center for Health Sciences, Memphis, TN 38163.

Two cell types - horizontal I and granule I cells - accumulate tritiated GABA injected into the cat superior colliculus (SC) (Mize, et al., J. Comp. Neurol., 206:180, 1982). We now report the pattern of SC labeling using an antiserum to GABA visualized by light and electron microscope immunocytochemistry. Fifty um sections were cut through the SC of 9 cats. Each section was incubated in varying dilutions of GABA antiserum (Immuno Nuclear) and reacted using the avidin-biotin (ABC) technique. At the LM level, neuropil labeling was most dense within the zonal and superficial gray layers, but labeling was also observed deeper where large, densely stained fibers coursed through the intermediate and deep gray layers and the colliculus commissure. Apparent labeled terminals surrounded large cells in the deep strata. There was a marked variability in staining intensity in different cells. Most intensely labeled cells were located in the zonal and superficial gray layers, but some were also found in the deep layers. Most but not all intensely labeled cells were small (mean aver. diameter = 13 um) and had a variety of shapes.

With the electron microscope we observed several types of labeled cell. The most common type was small with a round or ovoid cell body and cytoplasm clumped at one end. Another type had a horizontal, fusiform cell body with thin cytoplasm surrounding a prominent, invaginated nucleus. Occasionally, labeled cells with morphologies distinct from these two were also seen. We found no clearly labeled glia.

Four distinct varieties of labeled process were observed. Myelinated axons were commonly seen. Conventional dendrites with parallel arrays of labeled microtubules were also seen frequently. Some of these had a horizontal orientation. One labeled dendrite gave rise to an unlabeled spine which contained a cluster of flattened vesicles and synapsed onto another process. Two other varieties of vesicle-containing profile were labeled. One had loose accumulations of pleomorphic vesicles which were small and usually round or ovoid in shape. These often received input from retinal terminals and formed symmetric synaptic contacts with other processes. The other variety had densely packed pleomorphic vesicles, some of which were flattened. These profiles were always presynaptic to other profiles and also formed symmetric synapses.

Our results largely confirm those using uptake of tritiated GABA. However, the GABA antiserum reveals a richer variety of cells, some of which are located in the deep as well as the superficial subdivision of cat SC (Supported by EY-02973).

296.4 TIME COURSE OF ELECTROPHYSIOLOGICAL CHANGES IN THE SUPERIOR COLLICULUS OF ADULT LONG-EVANS RATS FOLLOWING MONOCULAR ENUCLEATION. W.K. Boyes and B. Veronesi. Neurotoxicology Division, U.S.E.P.A., Research Triangle Park, NC, 27711.

Previous electrophysiological studies (Fukuda et al., Jap. J. Physiol. 28, 347, 1978;) report that in the rat visual system, the faster conducting/larger caliber fibers of the optic tract terminate in the lower portion of the stratum griseum superficiale (SGS) of the superior colliculus (SC), whereas slower conducting/smaller caliber fibers terminate in the stratum zonale and the upper portions of the SGS. Degeneration studies (Lund et al., Brain Res. 116, 83, 1976) in contrast, based on the premise that small diameter fibers degenerate before large diameter fibers, conclude that larger diameter fibers innervate upper portions of the SGS, and smaller fibers the lower portion of the SGS.

To address this discrepancy, the present study examined the electrophysiological changes in the SC in response to monocular enucleation. Long-Evans, male, 60-day-old rats were implanted surgically with a chronic bipolar stimulating electrode located in the optic chiasm and bilateral monopolar recording electrodes located just below the SGS and referenced to a skull screw electrode. One week after surgery, baseline recordings were performed in awake rats. In rats with satisfactory electrode placements (n=17), peaks which corresponded to presynaptic activity of fast conducting fibers (Pre), postsynaptic activity of fast conducting fibers (N1), and postsynaptic activity of slow conducting fibers (P3), were identified based on peak latency, polarity, stimulation threshold and resistance to 100 Hz stimulation (Fukuda et al., Jap. J. Physiol. 28, 347, 1978; Wilson et al., Soc. Neurosci. Abs., 460, 1984). Two days after enucleation, the contralateral Pre and N1 peaks were almost undetectable, whereas P3 was still prominent. The P3 peak was reduced by 4 days, and undetectable by 7 days after enucleation. Enucleation produced no obvious ipsilateral changes. Morphological analysis of parallel-treated rats is in progress. The preliminary electrophysiological results suggest that following enucleation in adult rats, fast conducting optic tract fibers degenerate before slow conducting fibers. This finding could account for the discrepant conclusions of previous degeneration and physiological studies.

296.5 CORRELATIONS BETWEEN STRUCTURE AND FUNCTION FOR SINGLE VISUAL CELLS IN THE SUPERFICIAL LAYERS OF THE HAMSTER'S SUPERIOR COLLICULUS. R.W. Rhoades, B.G. Klein and R.D. Mooney. Dept. of Anatomy, UMDNJ-School of Osteopathic Medicine and Rutgers Medical School, Piscataway, NJ 08854.

Intracellular recording, receptive field mapping and HRP injection techniques were used to determine the structural and functional characterisitics of 59 cells in the superficial laminae (the stratum griseum superficiale-SGS and stratum opticum-SO) of the hamster's superior colliculus. Of these, 8 were marginal cells, 14 had stellate morphology, 10 were narrow field vertical cells, 12 were widefield vertical cells and 8 were horizontal cells. Seven had somadendritic morphologies which did not fall into any of these groups.

There were strong correlations between the structural and physiological characteristics of the recovered neurons. Sixty-four % of the stellate cells and 75% of the marginal cells were directionally selective. Only 17% of the other cell types exhibited this response property. In addition, only 36% of the stellate and 25% of the marginal cells were discharged by stationary flashed spots. Eighty-one % of the other cell types gave reliable responses to such stimuli. Only 29% of the stellate and 13% of the marginal cells responded to stimulus speeds in excess of 20°/sec. Seventy % of the other cells were reliably discharged by speeds >20°/sec. None of the receptive field properties which we evaluated functionally differentiated narrow field vertical, widefield vertical and horizontal cells.

Reconstruction of the axonal arborizations of the recovered neurons demonstrated further that some marginal, narrow field vertical and widefield vertical cells had axon collaterals which terminated in the deep collicular laminae. While most of these innervated the stratum griseum intermediale, those of several cells extended as far as the stratum griseum profundum.

Our results thus demonstrate that marginal and stellate neurons in the SGS and SO can be functionally distinguished from other morphologically defined cell types in these layers and further that some visual cells in the superficial layers innervate the deep tectal laminae.

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296.6 SELECTIVE SUPPRESSION FROM VISUAL STIMULI OUTSIDE THE CLASSICAL RECEPTIVE FIELD OF NEURONS IN MONKEY SUPERIOR COLLICULUS. R.M. Davidson* and D.B. Bender* (SPON: M. Titmus). Div. Neurobiology, Dept. Physiology, Sch. of Med., Univ. at Buffalo, Buffalo, NY 14226.

In monkey extrastriate cortex, visual stimuli presented well outside the classically defined receptive field can radically influence the response to stimuli presented inside the receptive field. This "surround" influence can be suppressive and stimulus specific: maximum suppression occurs when the surround stimulus matches the stimulus in the classical receptive field (CRF) along some particular stimulus dimension, such as direction of movement. Neurons making these local-global comparisons may contribute to the segregation of figure from ground.

To determine whether neurons in the monkey superior colliculus also make such local-global comparisons, we examined the effect of moving surround stimuli on colliculus receptive field properties. Cells in both superficial and intermediate layers were studied in anesthetized, immobilized Macaca fascicularis. We first plotted the CRF using small spots of light, both moving and stationary. Almost all cells were non-directional, giving strong responses for all directions of movement. We then paired movement of a small spot across the CRF with movement of a very large "background" stimulus in the surround. The background stimulus was an annular array of 1 deg spots extending well into the peripheral visual field; the inner border of the annulus was no closer than 2 receptive field diameters to the CRF. The background spots always moved coherently. Four to 8 directions of background movement, spaced 45-90 deg apart, were paired with each direction of CRF stimulus movement. The background stimulus presented alone did not affect discharge rate.

All cells more than 300 microns below the tectal surface were powerfully influenced by movement of the background stimulus. When background and CRF stimuli moved in the same direction at the same speed, the cell's response was suppressed more than 60% below that evoked by the CRF stimulus paired with a stationary background. When the directions of target and background movement differed by 45 deg or more, there was little or no suppression. This pronounced and sharply tuned sensitivity to the relative directions of target and background movement held for every direction of target movement tested. The surround influence was less pronounced for cells in the superficial layers: for about a third of these cells, the response to the CRF stimulus was virtually unaffected by background movement.

Thus monkey colliculus neurons appear to make local-global comparisons based on differential movement, a process well-suited to the segregation of target from background.

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296.7 INTERMEDIATE GRAY OF MOUSE SUPERIOR COLLICULUS: CYTOCHROME OXIDASE RESOLVES LAMINAR BORDERS, PATCHES AND A FLANK WITH NO SUPERFICIAL GRAY LAYER. S.I. Wiener and P.H. Hartline. Eye Research Institute of the Retina Foundation, 20 Staniford St., Boston, MA 02114.

In order to identify anatomically the laminar architecture of the mouse superior colliculus (SC), the cytochrome oxidase (CO), acetylcholinesterase (AChE), myelin and Nissl stained sections were compared. In some cases, anatomical findings were supplemented by electrophysiological data; recording sites were identified by electrolytic lesions.

The CO and myelin stains resolve the SC laminae more clearly than does the Nissl stain. Myelinated fiber bundles, which define laminar borders explicitly, are recognizable in CO-stained sections by virtue of their weak staining. A prominent layer of large, rostrocaudally oriented fiber bundles occurs in a layer above the dorsalmost of the large multipolar cell bodies. This fiber layer has been identified in mouse and rat atlases as the intermediate white layer. But this seems to conflict with the accepted nomenclature for the cat SC and we suggest that this fiber layer be considered, instead, a subdivision of the intermediate gray layer. Such a change in nomenclature seems further warranted by the fact that the cat's intermediate white layer contains mediolaterally, not rostrocaudally, oriented fibers. Moreover, in the mouse, we find that AChE patches span both sides of the layer of rostrocaudally oriented fibers. In the cat SC, such patches occur only in the intermediate gray layer, whereas the earlier rodent nomenclature places them in both the intermediate and deep gray layers.

Patches of CO activity measuring 60-100 μm wide are found in the intermediate gray layer. In horizontal sections, the CO patches are seen to connect and form a meshwork. The patches extend dorsal and ventral to the layer of rostrocaudally oriented fibers, but are more intensely stained ventrally. Intensely CO-active neurons with large somata (5-20 μm diameter) aggregate into clusters which lie exclusively in the ventral aspects of the CO patches. There appears to be no systematic correspondence between these patches and those in the AChE stain as judged by a careful comparison of serially adjacent sections. The CO patches extend further dorsal and ventral and are thicker than the AChE patches.

The CO, myelin and AChE stains and electrophysiological recordings all indicate that the intermediate gray layer extends considerably further laterally than does the superficial gray. Single and multiple unit recordings in this lateral 'flank' region of the intermediate gray indicate that it receives peri-oral, and to a lesser degree, limb and trunk somatosensory as well as auditory inputs. These somatosensory inputs are continuous with the somatotopic map previously described for the more medial parts of the intermediate gray layer. Since the body regions represented in the flank are not in the visual field, the lack of overlying visual projections is in accord with spatial register.

296.8 SUPERNORMALITY OF RETINOTECTAL FUNCTION IN 2,5-HEXANEDIONE (2,5-HD) DISTAL AXONOPATHY OF THE SUPERIOR COLLICULUS BRACHIUM (BSC) IN THE HOODED RAT. D. Impelman*, R.D. Wilson* and D.A. Fox. (SPON: L.P. Felpel). College of Optometry, University of Houston, Houston, TX 77004 and USDA, College Station, TX 77801.

The 2,5-HD axonopathy of the BSC (Neuropath. Appl. Neurobiol. 8: 289, 1982) preferentially affects the functional properties of the $t_2$ (middle) conduction group in the optic tract (OT) (Neurosci. Abs. 10: 460, 1984). Field potential recordings were obtained simultaneously from presynaptic $t_2$ axons in BSC and postsynpatic $c_2$ cells in stratum griseum of the anterior SC (the SC dipole field response in rat). They show that the axonopathy produces supernormality (> 100% recovery) with a subsequent loss of postsynaptic inhibition in $c_2$ recovery functions and, in some animals, prolonged supernormality in $t_2$ functions. The distal-proximal gradient of supernormality in $t_2/c_2$ recovery functions suggests that (1) a functional gradient of supernormality in $t_2$ axons develops with the distal-proximal progression of morphological changes which characterize the axonopathy (i.e., neurofilament accumulation, axonal swelling and paranodal demyelination) and (2) the supernormality of $c_2$ and its loss of postsynaptic inhibition is attributable to hyperexcitability of OT preterminals in the SC of 2,5-HD animals. We measured the distal-proximal gradient of $t_2/c_2$ excitability by recording $t_2$ recovery functions in BSC and 5mm anterior in OT, and $c_2$ recovery functions in SC. Axons at the BSC recording sites were examined to correlate stages of axonal pathology with $t_2$ supernormality. The recordings showed a gradient of supernormality ranging from 107% in $t_2$ axons in BSC to 129% in $c_2$ responses in SC. However, OT $t_2$ axons recovered to 94%. The most notable difference between axonal populations at BSC recording sites of $t_2$ supernormality was the almost complete absence of medium diameter ($t_2$) axons with a concomitant increase in the number and heterogeneity of swollen axons. No signs of neuronal degeneration were observed. The effects of $t_2$ supernormality on intracollicular inhibition which normally depresses the recovery of $c_2$ responses 52-65% was studied by generating supernormality in $t_2$ recovery functions and simultaneously recording $t_2/c_2$ responses in control animals. Both functions show supernormal periods in good temporal correlation with approx. 15% more excitability in $c_2$ than in $t_2$ which is similar to responses in 2,5-HD rats. Intracollicular inhibition in these animals was depressed approx. 20% as compared to approx. 40% in 2,5-HD animals. Thus $t_2$ supernormality can produce $c_2$ supernormal periods in 2,5-HD animals. The larger subsequent loss of postsynpatic inhibition may involve transsynaptic effects of 2,5-HD as well as preterminal hyperexcitability.

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296.9 SUPERIOR COLLICULAR CORRELATES OF PARTIAL OPTIC TRACT LESIONS IN ADULT RATS. A.P. Foerster. Dept. of Neurosciences, McMaster University, Hamilton, Ontario, Canada L8N 3Z5.

The right superior colliculus (SC) of Long Evans rats (pentobarbital-anaesthetized) was mapped in 500 μm steps by transcortical penetrations, with tungsten-in-glass microelectrodes, to the depth at which the multiunit responses to a visual stimulus were optimal, and the "chug", a pentobarbital-related retina-dependent rhythmic bursting (15-20/s) was also maximal. At each SC site the location in the visual field, and the approximate size of the receptive field, of the multiunits were determined from the motion of a black card against the lighted background of a transparent hemisphere, r=34 cm, centered on the left eye. A dorso-ventral cut extending to the floor of the skull was then made by lowering a rectangular razor-ended blade, 1-2 mm wide, through the brain to cut the optic tract (OT) as it begins its ascent towards the lateral geniculate bodies; the blade was then withdrawn and the SC sites retested. When the SC was partially silenced the unresponsive sites (even to the motion of a very bright light in darkness) occupied a discrete area in which the chug was also absent. Intraocular HRP, and a sensitive glucose oxidase/TMB technique which demonstrates abundant reaction product with little crystalline artefact, revealed that the OT had been partially cut and that the terminal projections of the spared axons occupied the still-responsive region of the SC; they also extended into about 1 mm of the silenced area. Recordings made 3 hours - 3 days after such partial OT cuts occasionally revealed a return of response at a few sites within this innervated boundary zone, suggesting a recovery of conduction within a few initially conduction-blocked OT axons. Studies of retinas backfilled by injections of tracers into the SC support the anterograde and physiological findings.

Conclusions are: 1) The OT in the rat is retinotopically organized (cf. Bunt & Lund (1982). Soc. Neurosci. Abs. 8:451); 2) Conduction block, and subsequent recovery, of spared optic axons is a minor consequence of this lesioning technique; 3) Whether the silent fringe of optic innervation adjacent to a responsive region represents (i) a subthreshold excitatory input, (ii) a region of responses too small and/or sparse to be detected, or (iii) an inhibitory input, remains to be determined; 4) Since remaining multiunit receptive fields have normal sizes, sprouting or unmasking seem not to have occurred for at least 3 days; 5) This preparation is of potential value in analysing recoveries from OT injury, and in particular the question of whether these could be attributable to the regeneration of severed OT axons.

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296.10 DUAL TECTAL EFFERENT SYSTEMS FOR THREAT AVOIDANCE IN FROGS CORRESPOND TO TWO DISTINCT STIMULUS CONDITIONS. D.J. Ingle, Eye Research Institute, 20 Staniford St., Boston, MA 02114

A simple model of the frog's tectal efferent system is that turning towards prey is mediated by the crossed projection to brainstem and spinal cord, while turning away from threat depends only upon the uncrossed tectal output to the ipsilateral brainstem. Although these predictions have been confirmed by transecting either the tectal efferent decussation of the tegmentum or the ipsilateral efferents via a post-tectal hemisection, an unpredicted result of the second lesion is that frogs now respond to looming black disks in the contralateral field by jumping towards them! These "misdirected" avoidance responses were abolished by adding a tegmental split to the hemisections.

What could be the adaptive purpose of a crossed pathway, contributing to wrong-way avoidance jumps? Further cine analysis revealed two different avoidance strategies in normal frogs: (a) the familiar tendency to jump away from off-center threat on a collision course by turning into the contralateral field, and (b) a newly discovered ipsilateral field route elicited by stimuli about to cross the rostral midline a few inches in front of the animal. Reconstruction of the sequence via single-frame analysis showed that the ipsilateral jumps were determined by the stimulus trajectory rather than its radial position just prior to a given response. The same behaviors are observed in monocular or unitectal frogs. Thus a single eye-tectum system can program jumps in either direction depending on the direction of stimulus motion.

296.11 THE TRANSNEURONAL TRANSPORT OF HORSERADISH PEROXIDASE IN THE VISUAL SYSTEM OF THE FROG, RANA PIPIENS. Thomas E. Hughes* and William C. Hall (SPON: M. Gruenthal). Dept. of Anatomy, Duke University, Durham, N.C. 27710

During the course of experiments designed to study the synaptic relationships between the terminals of retinal axons and the various cell populations in the optic tectum of the frog, Rana pipiens, we have found that neurons in many of the retinorecipient nuclei, including the tectum, are transneuronally labeled following injections of horseradish peroxidase (Sigma, type VI) into the optic nerve. These neurons are well labeled; both the dendrites and the somas are filled with reaction product. However, unlike normal retrograde transport, the labeling is often heaviest in the dendrites that extend into the retinal recipient neuropil while the cell somas are only faintly labeled. Transneuronal transport is seen consistently when we use an imidazole enhanced diaminobenzidine reaction (Straus, J. Histochem. Cytochem. '82), and a 4 to 9 day survival period.

One of our most interesting findings is that particular cell groups in the tectum are well-labeled while other cell types, which on the basis of the location of their somas or dendrites seem equally likely to receive direct retinal projections, remain free of label. Electron microscopic investigation of the optic tectum reveals that the label is confined to pre- and postsynaptic processes. These results suggest that transneuronal transport in this system depends on a transfer of label from presynaptic terminals to postsynaptic cells rather than on a widespread diffusion of label through the neuropil followed by a selective uptake by particular cell groups. Further, the results suggest either that only some of the tectal cell groups which receive direct retinal projections are transneuronally labeled, or that only a few of the cell types in the tectum are directly contacted by retinal axons.

Although not all retinorecipient neurons may be labeled in this process, the transneuronal transport of horseradish peroxidase is useful since it reveals the morphology as well as the location of at least some of the retinorecipient cells. (Supported by N.I.H. grant EY-04060 and N.S.F. grant BNS-8109794 to W. C. Hall).

296.12 LOSS OF VISUALLY-GUIDED BEHAVIORS FOLLOWING ABLATION OF NUCLEUS ISTHMI IN THE FROG RANA PIPIENS. H.S. Caine* and E.R. Gruberg. Dept. of Biology, Temple University, Philadelphia, PA 19122.

Nucleus isthmi receives input from ipsilateral optic tectum and projects bilaterally to the optic tectum. Electrolytic ablation of the frog's nucleus isthmi results in a visual scotoma contralateral to the lesion. The extent of the scotoma has been determined by analysis of videotaped behavioral responses to visually presented stimuli. Within the scotoma, animals do no respond to visually-presented prey (live crickets tethered on a thread) or threats (a large black square suspended on a 1m rod). The size of the scotoma is related to the amount of n. isthmi ablated. The more extensive the lesion, the larger the scotoma. With complete unilateral ablation, a frog displays no visually elicited prey-catching or threat-avoidance behaviors in the entire monocular field. This specific visual loss is stable over time with the longest period of testing having lasted 8 months. Frogs with extensive bilateral electrolytic lesions exhibit scotomas with the same specific visual deficits as unilaterally lesioned frogs. These scotomas extend over the entire visual field. After 3-5 weeks there is some recovery of function within the binocular field, but no recovery is seen in the monocular fields.

The frog's posture and ability to move in any direction are uncompromised by n. isthmi ablation. Therefore, the behavioral deficit caused by n. isthmi ablation appears to be "visual" and not "motor". The scotoma that follows n. isthmi ablation appears to be identical to the behavioral effect induced by unilateral tectal lobe ablation (Ingle, D., Science, 181:1053, 1973). Unilateral transection of the forebrain at the diencephalon-telencephalon border contralateral to a n. isthmi lesion does not alter the scotoma induced by the n. isthmi lesion. Outside the scotoma, prey-catching and threat-avoidance are normal. Other visual functions including barrier-avoidance, scototaxis and optokinetic nystagmus are normal throughout the entire visual field. Supported by NIH Grant EY 04366.

296.13 GABAergic NEURONS COMPRISE A MAJOR CELL TYPE IN RODENT VISUAL RELAY NUCLEI. R.A. Giolli, G.M. Peterson, C.E. Ribak, M.H. McDonald, R.H.I. Blanks and J.H. Fallon. Depts. of Anatomy & Surgery, Univ. of Calif. Coll. Med., Irvine, CA 92717.

The enzyme glutamic acid decarboxylase (GAD) has been localized in sections of rodent brains (gerbil, rat) using conventional immunocytochemical techniques. The findings show that large numbers of GAD-positive neurons and axon terminals (puncta) are present in the visual relay nuclei of the pretectum and the accessory optic system. The areas of highest density of these neurons are the nucleus of the optic tract (NOT) of the pretectum, the dorsal and lateral terminal accessory optic nuclei (DTN, LTN), the ventral and dorsal subdivisions of the medial terminal accessory optic nucleus (MTNv, MTNd), and the interstitial nucleus of the posterior fibers of the superior fasciculus (inSFp).

The findings indicate that 27% of the NOT neurons are GAD-positive and that these neurons are distributed over all of the NOT except the most superficial portion of the NOT caudally. The GAD-positive neurons of the NOT are statistically smaller (65.9 $um^2$) than the total population of neurons of the NOT (84.3 $um^2$) but are otherwise indistinguishable in shape from the GAD-negative neurons. The other visual relay nuclei that have been analyzed (DTN, LTN, MTNv, MTNd, inSFp) are similar in that from 21% to 31% of their neurons are GAD-positive; however, these neurons are smaller in diameter and are statistically more spherical than the total population of neurons. This study further shows that a large proportion of the neurons in these visual relay nuclei are contacted by GAD-positive axon terminals. Although it is not possible to determine the exact number of all of these "GAD-recipient neurons", it is estimated that about one-half of the neurons of the NOT and the terminal accessory optic nuclei are opposed by seven or more GAD-positive puncta. Further, the morphology of the GAD-positive neurons combined with their similar distribution to the GAD-recipient neurons suggests that many of these neurons are acting as GABAergic, local circuit neurons. On the other hand, the large number of GAD-positive neurons in the NOT and MTN (20-30%) in relation to estimates of projection neurons ( 75%) presents the possibility that some may in fact be projection neurons. The overall findings provide morphological evidence which supports the general conclusion that GABAergic neurons play a significant role in modulating the output of the visually related NOT and terminal accessory optic nuclei. (Supported by USPHS grants EY03642, NS15669, NS20228, EY03018 and NS15321).

296.14 ON/OFF ORGANIZATION OF PATHWAYS SUBSERVING THE PUPILLARY LIGHT REFLEX. Andrew G. Knapp* and Peter H. Schiller. Department of Psychology, M.I.T., Cambridge MA 02139.

In mammals, the neural substrate of the pupillary light reflex includes both cortical and subcortical pathways. Evidence for cortical involvement comes mainly from studies in which electrical stimulation of occipital cortex (both within and outside of striate cortex) has been shown to elicit pupillary constriction. Anatomical and physiological evidence implicates the pretectal olivary nucleus (PO) in the subcortical pathway. PO receives a direct retinal projection and projects to the Edinger-Westphal nucleus, the site of preganglionic pupilloconstrictor neurons. Trejo and Cicerone (*Brain Res.* 300: 49, 1984) have demonstrated that electrical stimulation of PO in the rat evokes constriction of the contralateral pupil at low threshold, and that PO in this animal is made up largely of neurons that respond to retinal illumination with a sustained increase in firing rate (tonic ON cells).

As part of an effort to characterize the ON/OFF organization of the mammalian visual system, we have examined the direct pupillary light reflex of macaque monkeys and of rabbits following administration to the retina of 2-amino-4-phosphonobutyrate (APB), a drug that eliminates light responsiveness in ON bipolar cells and that has been shown to silence ON retinal ganglion cells in several species. Monkeys were maintained on light barbiturate anesthesia and were paralyzed to eliminate eye movements. Alert rabbits were restrained throughout the experiments and briefly anesthetized with halothane to receive intravitreal injections of APB (50-75 $\mu$l of a 10-20 mM solution). These injections were successful in eliminating retinal ON activity as inferred from complete abolition of the b-wave of the electroretinogram (Knapp and Schiller, *Vision Res.*, 24: 1841, 1984). Retinal illumination was provided though a fiber-optic light guide and pupillary reactions measured using a television pupillometer (kindly provided by Dr. Robert Kenyon).

We have studied both normal monkeys, normal rabbits, and rabbits which had received large unilateral ablations of the occipital cortex 2-10 days previously. In monkeys, APB had no discernible effect on the pupillary light reflex. In normal rabbits, APB raised the threshold for pupillary constriction by 0.5 log unit and reduced by 20% the maximal constriction elicited by suprathreshold stimuli. Both these effects were larger (1.0 log unit, 40%) in animals with cortical lesions. Moreover, in rabbits with lesions, but not in normal animals, APB altered the temporal course of the light reflex, making it markedly more transient. Prior to administration of APB, pupillary constriction lasted the length of the stimulus and recovery to the initial pupil diameter commonly took 10 seconds or more. Afterwards, constriction lasted no longer than 5 seconds regardless of the length or intensity of the stimulus. In agreement with previous physiological investigations, these results suggest that ON-channel input to the subcortical reflex pathway is tonic in nature. However, retinal OFF cells are apparently capable of mediating sustained pupillary constriction, provided the occipital cortex is intact. The pathways subserving the residual transient light reflex in rabbits with cortical lesions following APB administration remain to be determined. Supported by EY00676.

296.15 A GOLGI STUDY OF THE VISUAL TEGMENTAL RELAY ZONE IN THE RAT AND RABBIT. K.M. Gregory, Y. Torigoe*, R.A. Giolli and R.H.I. Blanks. Depts. of Anatomy and Physiology, CSU Long Beach, CA 90840 and Depts. of Anatomy and Surgery, Calif. Coll. of Med., Univ. of Calif. Irvine, Irvine, CA 92717.

A portion of the ventral tegmental area of Tsai (VTA) termed the visual tegmental relay zone (VTRZ) is known to have projections and some physiological response properties similar to the adjacent medial accessory optic terminal nucleus (MTN). Although the VTRZ is a part of the visual pathways related to the accessory optic and oculomotor systems, the morphology of the neurons in this region is poorly described. Accordingly, the present analysis examines the form of neurons in the VTRZ in Golgi-Cox and Golgi-Kopsch impregnated tissue.

The neurons forming the VTRZ have a distinctive dendritic architecture. Three types of neurons are identified within the VTRZ: (1) bipolar neurons with long (400-500 um) primary and secondary dendrites oriented in the mediolateral plane of the VTRZ; (2) small-to-medium sized multipolar neurons with primary dendrites also oriented in the mediolateral plane of the VTRZ; (3) large multipolar neurons with long (300-400 um) primary dendrites and a few secondary dendrites. These large multipolar neurons are located in the more dorsal region of the VTRZ and have dendrites extending either dorsally into the parvocellular subdivision of the red nucleus or ventrally into the nucleus parabrachialis pigmentosus of the VTA. Dendritic spines are scarce proximally, but increase in numbers on the more distal dendrites. Overall, the staining character, somal size and shape, dendritic diameters and dendritic spine density of the VTRZ neurons resemble more closely the features of the neurons of the parvocellular subdivision of the red nucleus than nuclei of the ventral tegmental area. The most laterally placed neurons of the VTRZ appear to be continuous with the neurons forming the dorsal division of the MTN. In fact, the dendritic extensions of the VTRZ neurons into the MTN form the morphological basis for the binocularity of the VTRZ neurons. The distinctive morphology of the neurons comprising the VTRZ suggests that this population should be identified as a separate nuclear subdivision of the VTA, a finding which is consistent with the connectional and functional subdivision of this cell population as a visual relay group. (Supported by USPHS grants EY03642 to RAG and EY03018 to RHIB. YT is a recipient of NASA Research Associate Award #MAGW-70.)

296.16 DIRECTION SELECTIVE CELLS IN THE NUCLEUS OF THE OPTIC TRACT IN THE SQUIRREL MONKEY. K.P. Hoffmann, Abt. Vergl. Neurobiologie, Universität Ulm, Postfach 4066, D-7900 Ulm, GFR.

In a number of mammals (rat, rabbit, cat) direction selective cells in the nucleus of the optic tract (NOT) have been shown to encode retinal slip and to be involved in the control of optokinetic nystagmus. In all mammals studied so far, these neurons project to the dorsal cap of the inferior olive. We now have been able to show that the same pathway exists in a primate.

In 2 squirrel monkeys 100 nl of horseradish peroxidase (HRP) were stereotaxically injected into the inferior olive under ketamine anaesthesia 2 days before the recording session. On the day of recording the animals were reanaesthetised with ketamine, fixed in a stereotaxic frame, artificially respired with $N_2O/O_2$ (2 : 1) and paralysed. The pretectum was approached stereotaxically directly from above. Recording sites were marked by microlesion. At the end of the recording session animals were deeply anaesthetised with nembutal and perfused transcardially for HRP-histochemistry.

The neurophysiological properties of NOT neurons in the squirrel monkey resemble very much those in the cat. Neurons had huge receptive fields (40° x 40°) including the fovea. Large area random dot patterns were optimal stimuli although single spots were also effective. All 8 neurons recorded in the left NOT preferred movements from right to left and were inhibited by movements in the opposite direction. All were binocular and showed the same properties through both eyes. Velocities from 0.1°/s to more than 300°/s were effective to modulate the activity of NOT cells direction specifically. Optimal velocities were 60 - 80°/s.

The recording sites of these neurons were found in the same part of the mesencephalon where the cells, retrogradely labelled from the inferior olive were located. These cells were dispersed in the NOT and in the fibers of the brachium of the superior colliculus. Our results clearly show that in primates as in other mammals the NOT cells relay information about retinal slip to the inferior olive.

296.17 RECEPTIVE FIELDS OF NEURONS IN THE PRINCIPAL OPTIC NUCLEUS OF THE PIGEON. K.H. Britten* and D.H. Cohen (SPON: A.D. Carlson). Dept. of Neurobiology & Behavior, SUNY at Stony Brook, Stony Brook, NY 11794

The principal optic nucleus (OPT) is the avian homologue of the mammalian dorsal lateral geniculate nucleus. Using a variety of stimuli, we have studied the visual receptive fields (RF) of these neurons to determine if they segregate into functional classes and how these classes might relate to those of mammalian LGN. The stimuli included: 1) a grid of stationary, flashed spots for describing center response type and RF size and geometry; 2) moving spots for exploring directional selectivity; 3) counterphased sinusoidal intensity gratings presented at eight spatial phases for investigating linearity of spatial and temporal summation; 4) drifting gratings for determining spatial and temporal frequency-response tuning functions; 5) whole-field illumination for measuring visual response latencies and for generating intensity-response functions; and 6) optic nerve stimulation for measuring response latencies. Animals were anesthetized with $N_2O$ and local anesthetic.

A preliminary sample of over 100 neurons showed large, homogeneous RFs with a mean area of 145 square degrees. Most (72%) had on-centers, with the remainder having on-off- (23%) or off-center (5%) responses. Antagonistic surround responses were never elicited by either spot or annular stimuli. RFs tended to be located forward of the optic axis, and the larger RFs were located outside the region of binocular overlap. Retinotopy is at best crude, since there was no apparent relationship between electrode and RF locations. All cells responded best to spatial frequencies below .45 cycles/degree, with some showing a response roll-off at the lowest spatial frequencies. Responses to counterphased gratings were quite nonlinear, showing rectification at low spatial frequencies and harmonic distortion at high spatial frequencies. Most neurons showed broad temporal-frequency tuning, and the distribution of preferred frequencies had a mode at 4 Hz.

The distributions of most response properties appeared continuous and unimodal. The exception was response duration which was bimodally distributed; some cells showed distinctly transient responses, while others had sustained responses. However, these two types of cells did not differ in other respects. Also, two-dimensional scatter plots and contingency tables revealed no relationships between response measures.

Given these results, we favor the hypothesis that OPT neurons represent a single, rather heterogeneous group which does not obviously resemble either the X- or Y-cells of the mammalian LGN. In many respects they resemble mammalian W-cells.(Supported by NSF Grant BNS-8016396.)

296.18 IDENTIFICATION OF THE RETINAL GANGLION CELLS PROJECTING TO A PRETECTAL NUCLEUS, THE LENTIFORM NUCLEUS OF THE MESENCEPHALON (LM) IN CHICKEN. Stefan R. Bodnarenko, Olivia C. McKenna and Shulamith Levi*. Dept. of Biology, City College of CUNY, New York, N.Y. 10031

In birds, the LM, which is considered homologous to the mammalian nucleus of the optic tract, is known to respond to retinal slip, i.e., whole-field visual motion, and is responsible for mediating horizontal optokinetic nystagmus (OKN). We have examined in chickens the retinal ganglion cells (RGC's) that project to the LM, the topography of this projection within the LM and the distribution of these RGC's across the retina using the retrograde HRP tract tracing technique.

After iontophoretic injection of 30% HRP into the LM of seven 5-6 week old chickens, retrogradely labeled RGC's were found only in the contralateral eye and almost exclusively within the retinal ganglion cell layer. The cells, which appeared round or oblong, ranged in size from 5 x 8 μm to 12 x 30 μm, with most cells measuring 10 to 15 μm along their long axes; separate classes of cells could not be distinuished. The location of labeled cells in the retina after HRP injections into limited areas of the LM strongly suggested that the LM projection is retinotopically organized: the inferior temporal retina projects to the rostral LM, the central retina to the middle of the LM along its rostro-caudal and dorso-ventral axes, and the superior nasal retina to the caudal LM. This retinotopy is consistent with that reported by Ehrlich and Mark (JCN 223 1984). Three HRP injections which included almost all of the LM resulted in labeled RGC's that lay in an oblique band extending from the inferior temporal retina to the superior nasal retina. After any of these injections few labeled cells were found in the two other retinal quadrants, the superior temporal and inferior nasal.

The RGC's projecting to the LM share several characteristics with a separate population of RGC's that project to another avian visual nucleus, the nucleus of the basal optic root (nBOR) which, like the LM, receives retinal slip signals and mediates OKN. In both cases the RGC's project in a topographical manner onto their recipient nuclei and their RGC's are located principally in areas of the retina where high concnetrations of other RGC's are not found. This location of cells projecting to the LM and nBOR in portions of the retina where visual acuity is low is consistent with the findings that both nuclei respond to whole-field visual motion.

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297.1 IDENTIFICATION OF LOCAL NEURONAL CIRCUITS IN THE VISUAL CORTEX OF THE CAT. J.P.Landolt, S.Reinis and D.S.Weiss*, DCIEM, Toronto, and Dept. of Psychology, Univ. of Waterloo, Waterloo, Ont., Canada, N2L 3G1.

Local neuronal circuits in Brodmann's area 18** were analyzed following the recording of population neuronal activity by a single tungsten microelectrode. The receptive field areas, directional preferences, peristimulus time histograms, and spontaneous activity were identified both in the cell with the recorded spike having the largest amplitude ("leading cell"), and in the cell population surrounding the microelectrode. The spikes were divided into five classes according to their amplitudes. The correlation of the activities of individual classes of neurons (or even individual neurons) was then computed in the following way:

First, the auto- and cross-correlation histograms between and within individual amplitude classes of the spikes were computed. These histograms summarize the length and the frequency of all interspike intervals within the multiple cell record. Then, the most common interspike intervals were selected from each histogram. These latter intervals were attributed to the real pairs of spikes in the original records. All such pairs of spikes were listed in a separate file. Each pair was described by the position of the first and second spikes in the original record expressed in milliseconds from the beginning of the record; by the amplitude of the first and second spikes expressed in relative units; and by the interval between them. Using these data, a continuous system of neuronal spikes was constructed that was interconnected by the preselected intervals. In control calculations, records were constructed in which the numbers of the spikes were preserved, but their positions were randomized. No continuous systems could be constructed from the randomized data. In another set of calculations, the time-dependent distribution of spikes was collapsed, and the number of cells involved in the system of interactions was estimated.

The responses of the neuronal systems in the vicinity of the recording microelectrode were compared to the light bar moving in eight different directions across the receptive field of the leading cell. Although many cells in the recorded area responded to the visual stimulation, a change of the angle of the light bar altered their interactions. When the visual pathway was not activated by any defined visual stimulus, the activity of the neuronal systems was still present, and it was sustained by nerve impulses circulating in large reverberating circuits.

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**All experiments conform to guidelines approved by the Canadian Council of Animal Care.

297.2 DISCONTINUITY-ANALYSIS IN THE CAT'S AREA 19. H. Saito, K. Tanaka, Y. Fukada* and H. Oyamada*. NHK Science and Technical Research Labs., Setagaya-ku, Tokyo, Japan 157.

We studied receptive field properties of cells in the cat's area 19 using four animals anesthetized with gas mixture of $N_2O$ and $O_2$ (70:30-80:20) and paralyzed with gallamine triethiodide (7 mg $\cdot$ $kg^{-1} \cdot hr^{-1}$).

One of the most remarkable features of this area, found in this study, was that orientation selective cells which responded best to an elongated bar or edge of a particular orientation were in the minority (26/81, 32%). The majority (55/81, 68%) showed a good response to a non-oriented small stimulus moving in any direction. The minor group included cells which preferred long bars (longer than $32^o$) and cells whose responses were suppressed when the length of the bar exceeded a distance across the discharge center in both directions. The latter cells correspond to lower-order hypercomplex cells described by Hubel and Wiesel (J. Neurophysiol., 28:229, 1965).

For two-thirds of the major group of cells which showed the non-oriented responses, an elongation of the stimulus along an axis orthogonal to the direction of movement resulted in the suppression of responses. This suppression was observed for any direction of movement. These same cells, however, responded to a long bar if an end of the bar, i.e., a tongue was pushed into the discharge center from any direction. The response was again suppressed when the width of the tongue was increased.

To our surprise, a considerable number of cells which did not respond to a long bar moving across the receptive field in the direction orthogonal to its long axis, were activated by the same bar when just the discharge center was masked from the stimulation. Thus, the trigger feature for them is not only the presence of a small object, but also that of a discontinuous part of contours at a particular position in the visual field. This property cannot be explained by a simple model of the receptive field that the discharge center is surrounded by a pure inhibitory field. Instead, there may be some kind of nonlinearity in the inhibitory mechanism and/or a particular spatial arrangement of on- and off-structures in the organization of the receptive field of these area 19 cells.

The role of the area 19 contrasts with that of the area 17 which seems to decompose the visual world into oriented contours. The area 19 analyzes the visual pattern in its various aspects including detection of discontinuity, orientation and length of contours.

297.3 A REASSESSMENT OF THE VISUAL CORTICAL AREAS OF THE CAT'S SUPRASYLVIAN SULCUS. H. Sherk. Department of Biological Structure, U. of Washington, Seattle WA 98195.

A clue to the functions of the multiple visual cortical areas found in the cat may come from an understanding of how information is routed among them; the initial aim of this study was therefore to determine the connectivity of the 9 visual cortical areas reported by Tusa et al. (Cortical Sensory Org. vol. 2, 1981) to lie in the vicinity of the suprasylvian sulcus. However, these experiments showed an unexpected discrepancy between the visual areas identified by Tusa et al., and those defined in terms of their connections. A visual cortical area is often defined as a region containing a partial or complete hemifield represenation, but one can instead consider it to be a region whose parts are all connected to the same sets of cortical and thalamic regions, with the magnitudes of such connections being similar throughout. When this definition was applied to the visual suprasylvian cortex, I found that some of Tusa et al.'s areas could be combined, while 3 of them were subdivided because different regions within them showed quite different patterns of connectivity.

Inputs to sites in the vicinity of the suprasylvian sulcus were examined by making small injections of wheatgerm agglutinin conjugated to horseradish peroxidase at physiologically identified locations. Retrogradely labeled cells were counted in each identifiable area of cortex, and in thalamic nuclei; the resulting data were assessed statistically using cluster analysis. Segments of some borders between cortical areas were identified autoradiographically by labeling their inputs from contralateral area 19's area centralis representation with 3H-amino acid.

Both cortical and thalamic patterns of cell labeling indicated that a single cortical area occupies most of the medial bank of the suprasylvian sulcus, much of its posterior bank, and a small fraction of its lateral bank. The Clare-Bishop area, as I call it, takes in portions of between 4 and 6 of the visual areas described by Tusa et al.: their areas PMLS, 21a, VLS, DLS, and possibly PLLS and 21b. Its extent was confirmed by examining anterograde transport from 33 physiologically-identified injection sites in area 17, since the latter was found to lack any significant input to areas adjoining the Clare-Bishop area.

Tusa et al.'s area 21a was found, on the basis of its connections, to be split between Clare-Bishop and a distinct area lying medial and posterior (area 21). Area DLS was likewise found to be divided between Clare-Bishop and a visual region on the lateral bank of the suprasylvian sulcus. Finally, area PMLS was split between the Clare-Bishop area and a visual region lying anterior and medial to it. In summary, a simpler scheme resulted in which the visual cortex of the suprasylvian sulcus was divided into only 5 discrete areas.

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297.4 THE UPPER VISUAL FIELD MAP IN THE CLARE-BISHOP AREA OF THE CAT. M. Ombrellaro* and H. Sherk (SPON: A. Hendrickson). Department of Biological Structure, U. of Washington, Seattle WA 98195.

In the cat, the medial and posterior banks of the suprasylvian sulcus can be considered to comprise a single visual cortical area because tracer injections anywhere in this region show the same pattern of extrinsic connections (see accompanying abstract). The Clare-Bishop area, as we call it, includes the lower visual field representation of area PMLS of Palmer et al. (JCN 177: 237-256, '78). Its upper field representation, if any, is not well understood, and the aim of this study was thus to determine the location and organization of this representation.

To do so, we made small anterograde tracer injections into physiologically identified sites in area 17's retinotopic map, and examined the resulting patches of label in the Clare-Bishop area. Two distinguishable tracers (3H-amino acid and wheatgerm agglutinin conjugated to horseradish peroxidase) were injected at different sites in each cat to allow visualization of the relative positions of two visual field loci. We also mapped the posterior Clare-Bishop area physiologically, and in the same cats injected the area centralis (AC) of area 17 with tracer to obtain both physiological and anatomical data in single experiments.

The upper visual field representation of the Clare-Bishop area was found to differ from those in areas 17, 18, and 19 in two obvious ways. First, the map was coarser: a given patch of visual field occupied a relatively larger fraction of Clare-Bishop. Second, this area contained redundant representations of a significant portion of the visual field. At the boundary between the upper and lower visual quadrants, the map contained two distinct AC representations, one on the lateral and the other on the medial margin of the Clare-Bishop area; they were separated by a short segment of horizontal meridian (HM). The vertical meridian (VM) bounded the Clare-Bishop area posteriorly. In the depths of the posterior suprasylvian sulcus, the area was bounded by the HM, which extended laterally from the lateral AC out to the far periphery's representation. The map was complicated by a forking of the VM: its central segment (from $0^o$ to about $+10^o$) had a duplicate representation that ran dorsoventrally down the face of the posterior suprasylvian sulcal bank to terminate at the lateral AC. Because this VC representation was embedded within the Clare-Bishop area, roughly the central $10^o$ of visual field must be represented at least twice, once on either side of the VM. The data suggest that in fact there may be three representations of this segment of visual field, with the most anterior one being quite compressed. We would conclude that, although its connectivity indicates that the Clare-Bishop area is indeed a single entity, within this area there are redundant representations of a portion of the upper visual field.

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297.5 BINOCULAR ACTIVATION OF ANTERIOR ECTOSYLVIAN VISUAL AREA (EVA) NEURONS IN SPLIT-CHIASM CATS. M.Ptito, G.Tassinari* and A.Antonini*. Groupe de Recherche en Neuropsychologie, Univ. du Quebec,CP 500, Trois-Rivières, PQ, CAN G9A 5H7 and Istituto di Fisiologia Umana, Strada Le Grazie, Verona, Italia 37121.

In the present experiment we have evaluated electrophysiologically the contribution of the corpus callosum (CC) to the receptive field properties of EVA neurons in split-chiasm (SC) preparations. Ten SC cats were used; in 6 of them the posterior half of the CC was sectioned during the actual recording in EVA ("acute") whereas in the remaining 4 the same portion of CC was cut 2 weeks prior to the physiological experiment ("chronic"). Single units responses were obtained with tungsten microelectrodes in anaesthetized, curarized preparations. The following stereotaxic coordinates were used to reach EVA: AP 11 to 14mm and L 12 to 14mm. Receptive field properties for each eye were studied before and after callosotomy using moving slits of light or shadows projected onto a screen 57 cm away from the animal's eyes.

Our results have shown that:

1) a very high proportion of EVA neurons (79%) in SC cats were driven by stimuli presented to either eye;

2) both receptive fields of each binocular neuron in SC cats abutted or were very close to the vertical meridian;

3) the receptive field size of EVA neurons was generally large (X=27°) thus providing for a wide representation of the ipsilateral visual field;

4) the majority of units (80%) encountered in EVA were bimodal (visuo-tactile) and the somatic receptive fields were often bilateral;

5) after the "acute" or "chronic" callosal section all neurons became monocularly driven whereas the bilateral somatic responses were unaffected.

These results led to the conclusion that EVA is a high order visual area whose neuronal properties are similar to those of the Lateral Medial Suprasylvian Area (PMLS) with which it has the richest anatomical connections. More importantly, our data indicate that the posterior part of the corpus callosum is an important contributor to the physiological organization of EVA neurons. This interhemispheric influence is likely to be mediated by a polysynaptic loop involving PMLS on both sides of the brain.

297.6 CORTICAL CONNECTIONS OF AREA 18 IN SQUIRREL MONKEYS. J. H. Kaas and C. G. Cusick. Dept. of Psychology, Vanderbilt University, Nashville, TN 37240.

Cortical connections in squirrel monkeys were investigated after single or multiple injections into area 18 of the anatomical tracer, wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP). In order to better visualize the areal patterns of connections, visual cortex was flattened and cut parallel to the surface. Brain sections were treated with tetramethyl benzidine for revealing WGA-HRP, or stained for cytochrome oxidase or myelin. All injections were placed in the portion of area 18 on the dorsolateral surface of the brain.

Single injections revealed punctate intrinsic connections in cortex immediately surrounding the injection site, and punctate foci of intrinsic connections spaced mediolaterally over 5 mm from the injection site. Interconnections were apparent in area 17, in cortex just rostral to area 18, and in the middle temporal visual area (MT). Additional features of these connections were revealed by a mediolateral row of injections that fused to form a 2 x 12 mm bandlike injection zone in caudal area 18. Such an injection band revealed short .2-.5 mm semiperiodic rostral extensions of intrinsic connections in medial area 18 devoted to the lower visual quadrant, and a more extensive 2 mm zone of rostral intrinsic connections in lateral area 18 representing central vision. In different experiments, dense connections in area 17 varied from forming a continuous lattice in upper layer III with the holes matching the locations of cytochrome oxidase dense blobs on adjacent sections, to punctate foci matching the location of of the blobs. A region of cortex rostral to area 18 was labeled with closely-spaced patches of label in a 2-3 mm x 6 mm strip of cortex that merged laterally with the intrinsic label in rostral area 18 and extended medially to curve rostrally away from the area 17-18 border. A 3-4 mm extent of unlabeled cortex separated the rostral extent of this strip from MT. The label in MT was sparse and patchlike. Callosal connections in caudal area 18 formed meshlike extensions into rostral area 18, and crossed the width of area 18 laterally. Patchlike callosal connections were present in cortex rostral to area 18. One of the implications of the results is that there are at least two visual areas between VII and MT.

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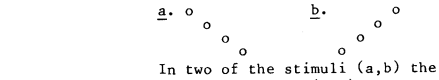
297.7 EVENT-RELATED CORTICAL FIELD POTENTIALS IN MONKEYS DISCRIMINATING PATTERN CATEGORIES THAT HAVE NO LOCAL FEATURE DISTINCTIONS. R.K. Nakamura, J. Johannesen* and R. Coppola, Laboratory of Psychology and Psychopathology, NIMH, Bethesda, MD 20205.

We have been recording event-related potentials (ERPs) via cortical surface-to-depth bipolar electrodes, in monkeys trained to perform a visual go/no-go discrimination task with reversals (Nakamura et al., Neurosci. Abs., 1984). To minimize artifacts related to eye movements or eye position, we have developed a new stimulus set that consists of four stimuli of four dots each:




In two of the stimuli (a,b) the four dots form diagonal lines, and in the other two (c,d) the four dots form diamonds with the same dominant angle as the diagonals. The objective for our monkeys is to respond in one way to the diagonals (e.g., go) and another to the diamonds (e.g., no-go). Brightness, contour, and distance of dots from any fixation point are inherently controlled and no single dot can be used to distinguish between the stimulus categories. The animals must base their responses on the spatial relationship of the dots. In addition, the stimuli are symmetrical so that there is no stimulus-generated inclination to move the eyes from the center of the stimulus presentation screen. With this stimulus set in our task it is possible to evaluate stimulus, stimulus category, motor response, and cortical area differences in the ERPs.

To date we have analyzed the task-related ERPs generated by this stimulus set from 32 electrodes in the cortical convexity of two monkeys. ERPs from all electrodes displayed additivity in that the early sensory components generated by the diagonal stimuli (averaged together) were identical to those generated by the diamond stimuli (averaged together) even though the individual stimuli produced highly differentiable results in some cortical areas. All electrodes in modality specific visual areas showed clear stimulus-related differences beginning at about 80 ms in striate cortex. The differences seen in striate and prestriate cortex could be related to the locations of the dots within the individual stimuli. Unlike striate and prestriate placements, two inferior temporal electrodes showed ERP differences between 160 and 200 ms that predicted the animal's motor response though time-locked to the stimulus, and preceded it by 140-180 ms. Contrary to prior reports, none of the visual system ERPs showed differences that were time-locked to the motor response.

Electrodes outside of the modality-specific visual areas showed only motor response-related differences, not stimulus-related differences. In prefrontal and parietal areas these differences appeared only after the motor response had begun.

297.8 DIRECTIONAL SENSITIVITY OF PARIETAL VISUAL NEURONS TO MOVING STIMULI DEPENDS UPON THE EXTENT OF THE FIELD TRAVERSED BY THE MOVING STIMULI.

B. C. Motter, M. A. Steinmetz, & V. B. Mountcastle, Bard Laboratories of Neurophysiology, Dept of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Parietal visual neurons display a lack of specificity for many physical properties of visual stimuli (e.g., orientation, color, speed of motion), but they are often quite sensitive to the direction of motion along any given meridian. We have investigated whether the directional sensitivity observed for simple bar stimuli during long traverses of the visual field is produced by local mechanisms encountered in each succeeding region of the visual field.

Monkeys (M. mulatta) were trained on a fixation/detection task during which visual stimuli irrelevant to the behavioral task were used to determine visual response properties. The sequential and cumulative aspects of directional sensitivity were examined in several different stimulus presentation paradigms, including: (a) decomposition of long stimulus movements into a series of shorter movements; (b) conditioning and test (C&T) paradigms designed to separate spatial and temporal sequencing effects; and, (c) simultaneous presentations of two stimuli in different areas of the visual field.

We found that for the majority of comparisons the directional response elicited from a local region (10 deg) of the visual field by stimuli restricted to that region could not account for the directional responses elicited from that same small region by stimuli passing through it while on much longer (90 deg) trajectories. Nevertheless, the C&T paradigms revealed that the directional sensitivity observed for the long movements required that stimulus passage be spatially continuous through regions adjacent to the test location, although the temporal C&T separation could be as large as 600 msec.

The studies with dual stimuli presented simultaneously revealed that responses to complex stimuli could not be derived by any linear combination of the responses to individual stimuli, although in some cases the response to the combined stimuli appeared to be predicted by the best response to a single stimulus. Often, stimuli that when presented alone evoked no response could alter the responses to other stimuli when presented simultaneously, with either facilitation or suppression. This indicated an effective convergence from very large regions of the visual field. Indeed, it may be that under certain stimulus conditions some parietal visual neurons may be influenced by stimuli throughout the entire visual field. (Research supported by USPHS, EY03167)

297.9 ATTENTIVE FIXATION INFLUENCES DIFFERENTIALLY THE RESPONSES OF VISUAL NEURONS OF PRESTRIATE AND PARIETAL AREAS OF THE CEREBRAL CORTEX.

M. A. Steinmetz, B. C. Motter, & V. B. Mountcastle, Bard Laboratories of Neurophysiology, Dept of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, Md 21205

The responses of parietal visual neurons are strongly facilitated during a state of attentive fixation of a small foveal target, when compared to the responses to identical stimuli delivered in an alert state without target fixation (Mountcastle, et al., J. Neuroscience, 1981). We have now compared the effect of attentive fixation upon neurons of the prestriate (V4) and parietal (PG) cortical areas, in the same animals and under the same behavioral and stimulus conditions.

Male monkeys (M. mulatta) were trained to achieve and maintain fixation of a small target light for 2-5 sec, and to detect its dimming for reward. During the fixation period visual stimuli were used to determine the functional properties of prestriate neurons, and to map their receptive fields. Optimal stimuli were then delivered, and the responses evoked by them were compared with those evoked by physically and retinotopically identical stimuli deliveredin the intertrial interval, during eye pauses made as the animal viewed - without attentive fixation - his visual environment, awaiting the next appearance of the target light. The strong facilitation (3x) of the responses of parietal visual neurons was regularly observed. By contrast, the responses of the population of prestriate neurons did not differ significantly in the two behavioral states.

Thus the effect of directed attention upon cortical neuronal excitability can be quite different in two closely related cortical visual areas. The resultimplies that the neuronal systems mediating the facilitatory effect of directed attention can do so in a rapidly varying and highly differential manner as regards different cortical areas. The present results on the prestriate cortex were obtained in theexposed portion of the prelunate gyrus, and do not preclude the possibility that directed attention may affect the excitability and response properties of neurons in other prestriate areas. (Research supported by USPHS, EY03167)

297.10 THE FUNCTIONAL AND ANATOMICAL SUBDIVISION OF THE INFERIOR PARIETAL LOBULE. R.M. Siegel, R.A. Andersen, G.K. Essick and C. Asanuma. The Salk Institute, La Jolla, CA 92037.

The identification of functional subdivisions within the inferior parietal lobule is a necessary step in determining its processing role in spatial perception, spatial orientation, and attention. In these experiments we have identified four cortical areas in the inferior parietal lobule and adjoining dorsal aspect of the prelunate gyrus of macaque monkeys on the basis of different functional properties and cortico-cortical connections: areas 7a, 7b, the lateral intraparietal area (LIP), and the dorsal prelunate area (DP).

Area 7a contains neurons responsive to visual stimuli and to eye position. The receptive fields are large and generally bilateral. The responsiveness of the retinal receptive fields of many of these neurons is influenced by eye position and this modulation produces an eye-position-dependent tuning for the location of visual stimuli in head-centered coordinates. Although there is no obvious retinotopic order of receptive fields in 7a, a crude topography for head-centered coordinate space was found in two hemispheres with upward positions represented more dorsally than downward positions.

Area 7b, located rostral to 7a on the convexity of the inferior parietal lobule, contains cells that are predominantly selective for somatosensory stimuli.

The lateral intraparietal area (LIP) is located on the caudal aspect of the lateral bank of the intraparietal sulcus (caudal half of area POa). It contains neurons sensitive to visual stimuli and eye position and has many more cells that respond to saccadic eye movements than does area 7a.

The dorsal prelunate area (DP) is located on the convexity of the dorsalmost aspect of the prelunate gyrus abutting area 7a rostrally and V4 ventrally. It provides a major visual projection to LIP and 7a. Its receptive fields are smaller than those found in 7a and LIP and are generally located in the contralateral inferior visual field.

Small injections of anterograde and retrograde tracers in these areas reveal that they have different patterns of reciprocal cortico-cortical connections; their only common connection is with area MST. Notable differences are that LIP, which contains a majority of the saccade-related neurons, projects to the superior colliculus and strongly to frontal eye fields, whereas area 7a has only weak connections with the frontal eye fields and is much more strongly connected to area 46 of Walker in the frontal lobe. Area 7b has connections with somatosensory areas (area 5 and insular cortex) and area DP has connections with extrastriate visual area V3A.

By comparing the laminar organization of the terminations and sources of the cortico-cortical projections it was possible to construct a hierarchy for the transmission of visual information from V1 to area 7a (which is at the top of the hierarchy). Several parallel paths exist for this flow to 7a, but all require relays through at least two other extrastriate visual areas.

297.11 ORGANIZATION AND NEURONAL PROPERTIES OF VISUAL AREA TEO. S.B. Fenstemaker*, T.D. Albright and C.G. Gross (SPON: B. Campbell). Dept. of Psychology, Princeton Univ., Princeton, NJ 08544.

In the macaque, the cortical region anterior to visual area V4 and posterior to inferior temporal cortex (IT) has been termed TEO. It is reciprocally connected with both V4 and IT and the laminar distribution of these connections suggests that TEO follows V4 and precedes IT in the hierarchy of visual cortical areas that begins in V1 (Fenstemaker et al., ARVO 25, 1984). Unlike in IT, receptive fields of TEO neurons are always unilateral, and unlike in V4 they are within a few degrees of the center of the visual field (Desimone and Gross, Brain Res. 178, 1979). In order to clarify further its organization, properties and extent, we recorded from single and multi-units in TEO and surrounding cortex in immobilized animals anesthetized with nitrous oxide.

We found a representation of the center of gaze immediately anterior to the ascending tip of the inferior occipital sulcus (IOS). This representation is contiguous with that in V4 and forms the posterior border of TEO. Moving anteriorly within TEO, there is a representation of the central 10° of the inferior quadrant of the contralateral visual field located between the lower lip of the superior temporal sulcus and the lateral lip of the occipito-temporal sulcus. This representation is not well organized topographically. That is, on some penetrations the receptive fields were in approximately the same location in the visual field, while on other penetrations, the receptive fields were widely scattered. In contrast, we found the representation of the visual field in V4 to be more clearly topographic. Furthermore, within TEO, the neurons are less selective for orientation and more selective for complex stimuli, such as textures, than are those in V4.

The border of TEO with IT cortex lies 6-8 mm anterior to the ascending tip of IOS. Approaching this border, receptive fields become larger and their centers approach the horizontal meridian. Crossing into IT cortex, the neurons have more complex stimulus selectivity and still larger receptive fields which usually extend into both the inferior and superior visual fields and across the midline into the ipsilateral visual field. Previously we reported that dorsal V4 projects more strongly to TEO than to TE (ARVO, 1984). The border between TEO and IT as defined by these projections coincides with that defined here by receptive field size, and location and stimulus selectivity.

297.12 MULTIPLE VISUAL AREAS IN THE CAUDAL SUPERIOR TEMPORAL SULCUS OF THE MACAQUE. L.G. Ungerleider and R. Desimone. Laboratory of Neuropsychology, NIMH, Bethesda, MD 20205.

Anatomical studies have shown that area MT projects to much of the cortex surrounding it within the caudal STS. Yet, the boundaries, visuotopic organization, and neural properties of areas outside of MT remain unclear, and there are even conflicting data on the boundaries of MT itself. To examine these issues, we recorded from neurons throughout this region in three monkeys and then injected anterograde or retrograde tracers into MT to identify its projection fields.

Several visual areas were distinguished on the basis of visual topography, neural properties, receptive field (RF) size, myeloarchitecture, and pattern of connections with MT. MT, defined as the heavily myelinated portion of the V1 projection zone in STS, contained a systematic representation of only about the central 30°-40° of the contralateral field. The far peripheral field was represented medial to MT in a zone we term MTp, which receives projections from far peripheral V1 and V2. Like MT, MTp contained a high proportion of directionally selective cells, and RF size in MTp was the size expected of MT fields if MT fields were to extend into the periphery.

Areas MST and PP were found medial to MT and MTp. Both MST and PP had a high proportion of directionally selective cells, but only MST received a direct projection from MT. MST had larger RFs than MT or MTp but nonetheless had a crude visual topography. RFs in PP were even larger, some including the entire contralateral visual field. Furthermore, unlike cells in MST, some in PP responded to auditory or somesthetic stimuli in addition to visual.

Area FST, which has a distinctive myeloarchitecture, was found in the fundus of STS anterior to MT. FST received a direct projection from MT, but only about 1/3 of its cells were directionally selective. RFs in FST were large, often included the center of gaze, and were often bilateral.

Area V4t and a portion of V4 were found lateral to MT within STS, and both received direct projections from MT. V4t has a distinctive, light myelination. Both areas had a low incidence of directionally selective cells, and both contained coarse representations of the lower visual field.

These results combined with those of other studies suggest that MT, MTp, MST, and PP, together with area STP, constitute a cortical system for motion analysis. As one moves through this system, neurons appear to integrate motion information over an increasingly large portion of the retina, respond selectively to more complex types of motion, respond to inputs from additional sensory modalities, and may become more directly involved in oculomotor control.

297.13 INFERIOR TEMPORAL NEURONS DO NOT SEEM TO CODE SHAPE BY THE METHOD OF FOURIER DESCRIPTORS. T.D. Albright, R.A. Charles*, and C.G. Gross. Dept. of Psychology, Princeton Univ., Princeton, NJ 08544.

Inferior temporal (IT) cortex is necessary for normal shape discrimination learning and many IT neurons are selective for shape. Any shape may be described by a set of Fourier Descriptors (FD's). This method of shape description is used in computer pattern recognition (Zahn and Roskies, I.E.E.E. Trans. Comput. 21, 269). Previously we suggested that IT cortex might code shape by this method. The FD method depends, first, on determining the boundary orientation function for the shape, i.e., the orientation (tangent angle) of the shape's boundary measured at regular intervals around the perimeter. Then, this function is expanded in a Fourier series. Each term in the expansion is associated with a particular frequency, amplitude and phase and is known as an FD.

If IT cells acted as filters or detectors for FD's, the activity of an ensemble of such cells could code any shape. To test this possibility, we studied the responses of IT neurons to stimuli that were produced by the inverse transforms of single FD's. This process uniquely determines a shape which has a specific number of lobes (corresponding to frequency), lobe indentation (amplitude), and orientation (phase) and is known as an FD stimulus. Previously, we showed that many IT cells were selective for the frequency and amplitude of FD stimuli and maintained this selectivity over changes in the size, contrast and retinal location (P.N.A.S. 80, 5776). In the present study, we further examined the possibility that IT cells might act as filters or detectors for FD's. After determining the selectivity of each IT cell for the frequency of FD's, we presented stimuli produced by combining two FD frequencies. If IT cells act as filters for FD's, then the responses to the combined stimuli should be predictable from the responses to the component FD stimuli. Of 135 visually responsive IT cells, 49% were selective for particular FD stimuli. However, for none of these did we find a consistent relationship between the responses to the combined and component stimuli. These results suggest that the selectivity of IT cells for shape is not based on the analysis of boundary curvature into its component Fourier Descriptors.

297.14 NEURAL PATHWAYS MEDIATING DISTANCE ESTIMATION IN THE GERBIL. C. G. Ellard* and M. A. Goodale. Dept. of Psychology, Univ. Western Ontario, London, Ontario, Canada, N6A 5C2.

In previous experiments (Ellard, C. G., Goodale, M. A., and Timney, B., Behav. Brain Res., 14: 29, 1984), we have shown that Mongolian gerbils (Meriones unguiculatus) executed a series of vertical movements of the head prior to jumping over a gap that varied in size from trial to trial. Quantitative analysis of the size and frequency of these head movements suggested that their purpose was to generate motion parallax cues that were useful in judging distance. These cues appeared to be especially important at long distances (20 cm or greater), suggesting that other cues, such as loom and stereopsis, were more useful at short distances.

In the present experiment, we trained gerbils on the same jumping task and then subjected them to either aspiration lesions of visual cortex (areas 17, 18a, and 18b), radiofrequency lesions of the superior colliculus, ibotenic acid lesions of the pretectum, or sham operations. Preoperatively, all the animals showed the same relationship between gap distance and the frequency and amplitude of vertical head movements. After surgery, however, the pattern of head movements and the accuracy of the jumps changed dramatically in two of the operated groups. While the pretectal animals appeared to show little change in their behaviour, the animals with lesions of visual cortex or superior colliculus showed a significant decrease in the accuracy of their jumps compared both to their preoperative performance and to the sham-operated control group. Animals with cortical lesions were less accurate than animals with lesions of the superior colliculus. The frequency of head movements increased in both these groups, but visual cortical animals made many head movements at all distances while collicular animals, like the sham operates, made more head movements at long distances than at short distances. The mean latency to jump was significantly correlated with gap distance in both the collicular group and the cortical group, indicating some sparing of the ability to discriminate distance. These results suggest that while lesions of any one of the major retinofugal targets leave some residual depth discrimination intact, the severity of the deficit in visuomotor performance in the jumping task may vary depending upon the site of the lesion. The results further suggest that the ability to utilize motion parallax cues may depend to a large extent upon neural processing carried out within cortical areas 17, 18a, and/or 18b.

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297.15 SIZE-THRESHOLD CHANGES AFTER LESIONS OF THE VISUAL TELENCEPHALON IN PIGEONS. W. Hodos, S. R. B. Weiss* and B. B. Bessette*. Dept. of Psychology, Univ. of Maryland, College park, MD 20742.

Pigeons were tested in a psychophysical procedure that determined the limits of their ability to detect differences in the sizes of circular stimuli. On average, normal pigeons were able reliably to discriminate an annulus with a 3.0-mm diameter from one with a diameter of 3.9 mm.

In the first experiment, pigeons with lesions of the ectostriatum that spared its medial 15% were unimpaired in their size-discrimination ability. In contrast, those cases in which the lesions involved both the medial and lateral regions of ectostriatum had elevated size-difference thresholds. A second experiment, which was a replication of the first, yielded the same results and provided the additional observation that destruction of the medial 15% of ectostriatum with little or no damage to the lateral regions had no measurable effect on size-difference thresholds.

The medial region of ectostriatum is part of the termination field of a second tectofugal pathway to the telencephalon. This pathway passes from the optic tectum to nucleus dorsolateralis posterior thalami (DLP) and then to the medial neostriatum and the medial ectostriatum (C. A. Kitt & S. E. Brauth, Neurosci.,7:2735, 1982). Single-unit recordings have indicated a role for DLP in the transmission of visual information to the telencephalon (P. D. R. Gamlin & D. H. Cohen, Soc. Neurosci. Abstr., Vol 8, p. 206, 1982).

A comparison of the present data with those of previous psychophysical studies of ectostriatum suggests that its medial region may play a role for the processing of information about the low spatial-frequency components of visual stimuli.

297.16 ORGANIZATION OF CORTICOGENICULATE PROJECTIONS IN THE TURTLE, PSEUDEMYS SCRIPTA ELEGANS. P.S. Ulinski, Dept. Anatomy and Committee on Neurobiology, Univ. Chicago, Chicago IL 60637.

Turtles have a dorsal lateral geniculate complex that receives a bilateral, retinotopically organized projection from the retina and projects to a visual area on the ipsilateral cerebral hemisphere. This area includes the region known as the pallial thickening and the lateral part of the dorsal cortex or D2. It has been known for some time that the visual cortex projects back to the geniculate complex, but the organization of these projections had not been studied.

Cortical cells giving rise to the corticogeniculate projections were identified using the retrograde transport of horseradish peroxidase (HRP) following injections in the geniculate complex. Both D2 and the pallial thickening are trilaminar structures. Layer 1 consists of a few scattered neurons, none of which were retrogradely labeled by geniculate injections. Layer 2 consists of many densely packed oval somata. Labeled somata were scattered in layer 2 throughout the areal extent of the pallial thickening and D2. Layer 3 is a thin layer that contains a small number of fusiform somata with their long axes oriented parallel to the ventricle. Many of these were labeled. Thus, morphologically distinct cells in layers 2 and 3 contribute to the corticogeniculate projection.

The trajectory of corticogeniculate axons was studied using the orthograde transport of HRP following cortical injections. These axons course laterally between the lateral cortex and anterior dorsal ventricular ridge. They pass through the striatum bearing very few varicosities, enter the lateral forebrain bundle and turn caudally to run in the ventral peduncle of the lateral forebrain bundle. Individual axons turn dorsally as they pass beneath the geniculate complex and enter the complex from its medial face, running from ventral to dorsal through the geniculate cell plate. Each axon bears varicosities and terminaux en passant at irregular intervals, but never forms traditional axonal arbors.

Earlier work from this laboratory has shown that the geniculocortical projections are not topologically organized, having a point-to-line organization instead. This study shows the corticogeniculate projection has a similar point-to-line organization such that a given cortical neuron can contact geniculate neurons that lie along a vertical meridian of the retinal representation in the geniculate complex. Supported by PHS Grant NS 12158.

297.17 AN INTACT VERTEBRATE VISUAL SYSTEM IN VITRO: VISUALLY EVOKED PYRAMIDAL CELL RESPONSES IN TURTLE CORTEX. A.R. Kriegstein, (SPON: J. Wine). Dept. of Neurology, Stanford University School of Medicine, Stanford, CA 94305.

Young specimens (3-4 cm shells) of red-eared turtles, (Pseudemys scripta) were cooled to 0°C, decapitated, and the brains were surgically removed with eyes and optic nerves attached and submerged in 22°C saline in a recording chamber. Incisions were made along the medial walls of the cerebral hemispheres, and each dorsal cortex was unfolded and pinned back to expose the ependymal surface. The dorsal cortex contains the primary visual cortex of turtles, and in this preparation the retino-geniculo-cortical pathways were preserved. Light flashes were provided by a photo stimulator (Grass Model PS2) and directed to the contralateral eye by a fiberoptic conduit. Experiments were performed on turtles because they have a unique ability to function normally under conditions of prolonged hypoxia. Stable intracellular recordings were obtained from pyramidal neurons and responses to visual stimuli could be observed for up to 6 hours in vitro.

The pyramidal cell response to a brief (10 μsec) flash of light consisted of early excitation (50-100 msec) composed of EPSPs that often triggered one or more action potentials (APs). A subsequent inhibitory phase (250-400 msec) consisted of discrete synaptic events associated with net hyperpolarization and an increase in input conductance. Averaging 3-5 responses obtained at each of a series of potentials between -50 and -90 mV allowed calculation of reversal values for the early and late responses of -50 and -70 mV respectively. This suggested that the excitatory response may be shunted by concurrent inhibition and that the hyperpolarizing response may be composed of summated $Cl^-$ dependent IPSPs. This hypothesis was tested by recording from cortical neurons with KCl-filled microelectrodes. During intracellular $Cl^-$ injection the light-evoked response gradually inverted to a depolarizing event that lasted 200-400 msec and fired a barrage of APs. The hyperpolarizing component of the flash response therefore consists of prolonged activation of $Cl^-$ dependent IPSPs. A similar response sequence consisting of early excitation and long lasting GABA-mediated inhibition has been observed in the isolated cortical slab in response to electrical shock of thalamocortical fibers.

These results correspond well with anatomical, histochemical, and physiological data suggesting that geniculo-cortical afferents contact dendrites on both pyramidal cells and GABAergic interneurons, and that the interneurons in turn mediate feed-forward inhibition onto pyramidal cells. The in vitro turtle visual system will therefore allow detailed cellular analysis of light-evoked cortical responses.

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## *DEVELOPMENT AND PLASTICITY: VISUAL SYSTEM PLASTICITY*

298.1 LOSS OF VISUAL RECEPTOR CELLS DURING EARLY POSTNATAL DEVELOPMENT: EFFECTS ON VISUAL BEHAVIOR IN THE RAT. Jane D. Shepherd and Lawrence A. Rothblat. Department of Psychology, The George Washington University, Washington, D.C., 20052

We have been investigating the effects of light-induced photoreceptor degeneration on visual behavior to determine whether the types of visual impairments resulting from receptor cell loss are dependent on the time period in the life of the animal when the damage occurs. In the present study atropine sulfate (1% ophthalmic solution) was used in conjunction with high intensity continuous illumination (400 ft-c) to produce retinal damage in pigmented rats either early (15-28 days of age) or later (49-51 day of age) in development. For both groups the thickness of the outer nuclear layer was reduced by 50%. Control groups included (1) rats reared in a normal lighting environment without atropine (C1); (2) rats exposed to high intensity continuous light without atropine (C2); and (3) rats which were atropine treated but reared in a normal lighting environment (C3). Changes in retinal morphology were not evident in any of the control animals. The effects of the treatments were assessed by the performance of the rats tested as adults on both complex and simple visual discrimination tasks. For the complex task the animals were required to discriminate between columns and rows of 5mm squares. For the simple tasks the animals were required to discriminate between solid black-white striations (6,2,1mm in width) and a solid grey field of equal luminance.

Rats which had experienced retinal damage during early development were profoundly impaired on the complex discrimination when tested as adults; all animals failed to reach criterion within 1800 trials. In addition, these animals demonstrated small, but significant impairments on the simple discriminations in comparison to both control animals and rats suffering retinal damage as adults. The complex discrimination task was learned by all rats which had experienced damage as adults, although they were significantly impaired ($\bar{X}$ trials to criterion=594) compared to control animals (C1=336; C2=345; C3=344). On the simple discriminations, the performance of rats with retinal damage produced in adulthood was not significantly different from that of controls.

The results of the present study show that visual deficits which follow photoreceptor loss are related to the age at which the damage occurs, as well as the complexity of the behavioral task used for assessment.

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298.2 FUNCTIONAL PARCELLATION OF ACCESSORY OPTIC SYSTEM REQUIRES VISUAL EXPERIENCE. Ximena Rojas*, Olivia C. McKenna and Josh Wallman, Dept. of Biology, City College of CUNY, New York, NY 10031

In chicks the principal nucleus of the accessory optic system, the nucleus of the basal optic root (nBOR) and nBOR dorsalis are functionally divided into two parts, one responsive to upward visual motion and the other to downward visual motion. Several lines of evidence argue that these subdivisions provide the visual input for optokinetic eye movements in these directions. In previous studies we have shown that this functional parcellation develops to a large degree postnatally, as shown by (1) differences in the regions labelled when 2-deoxyglucose (2DG) is injected during the viewing of upward and downward visual motion (here called localization), (2) differences in the direction of highest OKN gain, and (3) differences in the location and properties of directionally selective single units. The first two of these effects have been shown to be affected by deprivation of visual experience.

To study whether visual experience during a critical period is essential for the development of this functional parcellation, we have studied, in the same individuals, OKN in a variety of directions and 2DG uptake during vertical visual motion, both in normal and visually deprived chicks. By 7 to 10 days in normal chicks, there is differential localization of 2DG uptake within nBOR. Birds deprived of form vision for the first 3 weeks of life showed no 2DG localization in the nBOR and had poor non-horizontal OKN with an abnormal direction of highest gain. Birds with the same period of visual deprivation, but then allowed 3 weeks of normal vision, showed the same effects, although the overall OKN gain was normal.

We infer from these results that visual experience seems necessary for the functional parcellation of nBOR, rather than simply accelerating it, since after six weeks, three of them with visual experience, there is no localization, although normally it appears after 7-10 days. These same results also suggest the existence of a critical period for the influence of visual experience, since 3 weeks of vision is ineffective if delayed by 3 weeks. The effect of visual deprivation noted here seems specific to the AOS, rather than being a generalized visual or oculomotor dysfunction, since horizontal OKN, which uses other visual pathways, appears to be normal. Finally, preliminary results indicate that birds with 1 week of visual experience beginning at hatching and then deprived for 2 weeks showed the same pattern of no localization and abnormal non-horizontal OKN, suggesting that localization, once attained, may not be permanent, but may require continued form vision for its maintenance. (Supported by NIH EY03613 and EY02937).

298.3 VISUAL SPATIAL DISCRIMINATION IN GOLDFISH FOLLOWING OPTIC TECTUM ABLATION. R. E. Davis, B. E. Schlumpf and P. D. Klinger*. Mental Health Research Institute, Univ. of Michigan, Ann Arbor, MI 48109.

Goldfish optic axons project sparsely to various diencephalic and pretectal nuclei and massively to the optic tectum. Bilateral tectum ablation results in greatly decreased sensitivity to classically conditioned visual stimuli and blockade of whole body orientation responses to dorsal light, food objects and optomotor stimuli (Yager et al. Brain Res., 137, 267-275, 1977; Schlumpf and Davis, Neurosci. Abstr., 10, 1158, 1984; Springer et al. Brain Res., 128, 393-404, 1977) suggesting that in part the tectum is necessary for discrimination of images in space. We examined whether nontectal optic pathways can mediate a conditioned spatial discrimination by ablating the optic tectum in previously conditioned fish. Discrimination was measured with a classically conditioned branchial suppression response.

The visual conditioned stimulus (CS) consisted of a spot of red diode illumination (1600 cd/$m^2$) that was presented for 5 sec. The positive CS was reinforced with an electric shock (US). Intact fish were trained to a nasal x temporal ($N^+ \times T^-$) or right X left ($R^+ \times L^-$) discrimination in lighted tanks. The nasal lamp was at 30° and the temporal lamp at 90° to the longitudinal axis of the fish and near the horizontal optic meridian. The lamp subtended < 1° at an apparent viewing distance of 34 cm. Following discrimination training the fish received bilateral optic tectum ablation and weekly sessions of discrimination test trials in darkness as illumination over 0.2 cd/$m^2$ blocked responding. The fish were dark adapted for 2 hr prior to each postoperative session.

Nine of nine R x L fish responded from the initial postoperative session and the mean response in R and L trials showed strong discrimination. The N x T fish initially responded to both stimuli and did not discriminate between them. Discrimination was restored after the second postoperative week. Whether the N x T discrimination is strictly monocular is unclear. When both stimuli were conditioned to the shock, during the fourth postoperative week, the responses to N and T or to R and L were similar indicating that all could be perceived. The average 50% threshold of response was 7.1 and 6.7 cd/$m^2$ to N and T and 3.2 and 3.4 cd/$m^2$ to R and L, respectively. Transection of the optic nerves blocked responding.

These results indicate that nontectal optic pathways can mediate discrimination of right from left eye stimulation and of nasal from temporal field stimulation. The implication is that the optic tectum is unnecessary for learning or memory of a simple visual spatial discrimination.

298.4 EFFECTS OF ABLATION OF VISUAL CORTEX IN NEONATAL RABBITS ON RETINOFUGAL PROJECTIONS. E.H. Murphy, A.M. Grigonis, T. Hayden*, D. Tashayyod* and M. Wilkes*. Dept. of Anatomy, Medical College of Pennsylvania, Philadelphia, PA 19129.

Visual pattern discrimination is severely disrupted in most mammals by ablation of visual areas receiving projections from the dorsal lateral geniculate nucleus (LGd). If a visual cortex lesion is performed in the neonate, some sparing of visual discrimination occurs in the cat but not in the rabbit. We have been comparing the morphological consequences of this lesion in cats and rabbits in order to elucidate the re-organization of subcortical visual projections which might underly the sparing observed in cats and the absence of sparing in rabbits. In infant operated (IO) cats we have reported significant expansion of the retino-pulvinar projection, sparing of large neurons in the LGd and significant transneuronal degeneration of medium sized retinal ganglion cells. In rabbits we have reported no transneuronal degeneration of retinal ganglion cells. In the present study we have analyzed cell survival in the LGd, and modification of retinofugal projections in IO rabbits. Unlike cats, there is no significant sparing of any population of neurons in the LGd in rabbits. However, there is a significant expansion of projections of retinal ganglion cells to the lateral posterior nucleus and to the pretectal nuclei. The results indicate that surviving LGd cells may play a role in behavioral sparing. However, expanded retinofugal projections to alternate termination sites and survival of retinal ganglion cells do not necessarily promote behavioral sparing in this preparation.

298.5 EFFECT OF REMOVAL OF RETINAL INPUT ON THE DEVELOPMENT OF THE COLLICULOGENICULATE PROJECTION. J. K. Sutton* and J. K. Brunso-Bechtold. Dept. of Anatomy, Bowman Gray School of Medicine, Winston-Salem, NC 27103

The dorsal lateral geniculate nucleus (dLGN) of the tree shrew (Tupaia belangeri) contains six cytologically-distinct layers separated by interlaminar spaces. Each layer receives a dense retinogeniculate projection and each interlaminar space receives a dense corticogeniculate projection. Layers 3 and 6, which contain small cells with similar connections, receive a dense colliculogeniculate projection as does the interlaminar space between layers 4 and 5. Prior studies have shown that following bilateral enucleation at birth, cytological characteristics of the layers remain yet interlaminar spaces fail to form and corticogeniculate fibers project diffusely across the nucleus instead of to the laminar borders as might be expected. Since colliculogeniculate fibers project to two dLGN layers as well as to one interlaminar space, it is possible to determine whether the absence of retinogeniculate fibers affects the development of laminar and interlaminar projections similarly. Based on the corticogeniculate projection in bilaterally enucleated animals, the interlaminar projection of the colliculogeniculate fibers is unlikely to terminate at the border between layers 4 and 5; however, it is of interest to know whether the colliculogeniculate projection to layers 3 and 6 remains denser than to the other layers. We therefore injected horseradish peroxidase into the superior colliculi of adult tree shrews which had been bilaterally enucleated at birth when colliculogeniculate fibers project diffusely across the nucleus in a topographic band, but do not reach the medial border. We then studied the pattern of anterograde transport to the dLGN to determine the effect of bilateral enucleation on the maturation of the colliculogeniculate projection pattern. In experimental animals, colliculogeniculate fibers terminate as densely in the region of dLGN layers 4 and 5 as they do in the region of layers 3 and 6; however, as in normal adults, the projection avoids the region of layers 1 and 2. The colliculogeniculate projection pattern reveals some specificity of termination since the projection is restricted to the lateral portion of the dLGN even in the absence of the retinogeniculate fibers which normally terminate in the medial layers. Nevertheless, competition between colliculogeniculate and retinogeniculate fibers must also play a role in the development of the normal colliculogeniculate projection pattern, since the projection in bilaterally enucleated animals is not limited to the small cell layers as is the case in normal animals.
(Supported by EY05028)

298.6 PHYSIOLOGICALLY IDENTIFIED RETINOGENICULATE AXONS DEVELOP NORMALLY IN DARK-REARED CATS. P.E. Garraghty, D.O. Frost and M. Sur. Sect. of Neuroanatomy, Yale Medical School, New Haven, CT 06510.

The normal postnatal development of X- and Y-cell retinogeniculate axons in cats (Sur et al., Nature, 310:246, 1984) can be dramatically altered by manipulation of visual input, such as monocular lid suture (MLS, Sur et al., Nature, 300:183, 1982). These observations, in aggregate, have led to the hypothesis that X- and Y-cell retinogeniculate axons compete during development for synaptic sites in the lateral geniculate nucleus (LGN).

In the present experiment, we attempted to expand our understanding of the process of X- and Y-cell development by examining the terminations of single retinal axons in the LGN of cats reared from birth in the dark. When these cats were at least 12 weeks of age, optic tract axons were recorded, classified as X- or Y-cell, and then impaled and intracellularly injected with HRP. In 5 dark-reared (DR) cats, we have recovered 10 X- and 12 Y-cell axons which were sufficiently filled to permit the complete reconstruction of their terminal arbors in the LGN.

Dark-rearing has no effect on the terminal morphologies of either X- or Y-cell axons. As in normal cats, X-cell axons project to lamina A or A1, ipsilateral Y-cell axons project to lamina A1 and contralateral Y-cell axons to laminae A and C. Furthermore, X- and Y-cell axons are normal both in their numbers of boutons and the volumes of their terminal arbors.

We find this result surprising because dark-rearing reportedly leads to a loss of physiologically defined LGN Y-cells, albeit without a change in LGN cell sizes (Kratz et al., Science, 203:1353, 1979). Monocular lid suture, which also leads to a loss of Y-cells recorded in the LGN, causes a reduction of retinal Y-cell arbors from the deprived eye in the A laminae of the LGN. In particular, in MLS cats, contralaterally projecting Y-cell axons often lack lamina A terminations. Yet Y-cell axons (both contralaterally and ipsilaterally projecting) in the DR cats develop normally. We considered the possibility that we simply failed to recover any affected Y-cell axons in dark-reared cats, but this possibility seems unlikely ($\chi^2$, $p<.02$; based on a comparison of whether contralaterally projecting Y-cell axons in DR and MLS cats retained their lamina A terminations). We are left with the possibility that dark-rearing and monocular lid suture have profoundly different effects on the development of retinogeniculate axons, such that development proceeds normally in the complete absence of light but is severely affected by the diffuse light stimulation present with lid suture. Finally, these results raise the question of how the retinogeniculate physiological relay can be so disrupted in the Y-cell pathway that retinal ganglion cell terminations are apparently normal while relay cells are irretrievably lost.

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298.7 ASYMMETRY OF BILATERAL VISUAL CORTEX LESIONS EFFECT AMPHETAMINE'S ABILITY TO PRODUCE RECOVERY OF DEPTH PERCEPTION. David A. Hovda, Richard L. Sutton and Dennis M. Feeney, Ment. Ret. Res. Ctr., UCLA, Los Angeles, CA and Dept. of Psychology and Physiology, UNM, Albuquerque, N.M.

We have reported that amphetamine (AMP) and visual experience can restore binocular depth perception after bilateral ablation of areas 17 and 18 in the adult cat (Feeney & Hovda, Brain Research, In Press). We now report that adult cats with complete removal of areas 17 and 18 (including the lateral suprasylvian gyrus) are able to perceive depth at presurgical thresholds after administration of AMP provided the bilateral lesions are symmetrical. Depth perception threshold was defined as the distance between the shelves at which the cat achieved 7 successes in 12 trials. The shallow side was randomly determined for each trial to control for side preference. Beginning at the greatest displacement, 12 trials were conducted and if the animal scored 8 or more successes the shelves were advanced 2 cm closer and tested another 12 trails. This was continued until the animal scored fewer than 8 successes. The shelves were then separated at 0.6 cm increments until the animal scored more than 7 successes. After 3 baseline testing sessions the striate and extrastriate cortex were aspirated bilaterally under standard aseptic conditions in 17 adult cats (male or female, 1.8-4.0 kg). Beginning on day 4 postsurgery the cats were tested every other day out to 30 days postsurgery. At day 10 postsurgery the cats were randomly divided into two groups. An AMP group received single d-AMP (5 mg/kg, i.p.) injections on days 10, 14, 18, and 22 postsurgery. The remaining animals served as saline (SAL) controls. On injection days animals were tested on the visual cliff 3 hours after AMP or SAL administration. After surgery all cats were unable to solve the simplest visual cliff. The SAL cats showed no improvement in depth perception throughout the experiment. Only the AMP cats showed recovery of depth perception as measured on the visual cliff. However, cats that did not show signs of AMP intoxication were like SAL treated animals in that they showed no recovery of depth perception. Furthermove, AMP treated cats with asymmetrical lesions (more of one hemisphere damaged than the other) showed only mild, drug dependent recovery with the depth perception deficit returning after discontinuation of AMP treatment. Finally, AMP-treated animals that had symmetrical lesions recovered to baseline levels and this complete recovery endured throughout the experiment. We therefore conclude that for AMP to produce a complete and total recovery of binocular depth perception after bilateral visual cortex ablation, AMP intoxication must be obtained and the bilateral lesions must be symmetrical.

298.8 EFFECT OF $GM_1$ GANGLIOSIDE TREATMENT ON CORTICAL RECOVERY FOLLOWING MONOCULAR DEPRIVATION IN KITTENS. G. Carmignoto*, R. Canella* and S. Bisti*° (SPON: G. Toffano). Fidia Neurobiological Research Laboratories, Abano Terme, Italy and °Institute of Neurophysiology, CNR, Pisa, Italy

Previous reports from our laboratory have described that $GM_1$ ganglioside treatment in kittens subjected to monocular deprivation is capable of partially preventing the ocular dominance shift of striate cortex neurons (Carmignoto, G., et al., J. Neurosci. Res., 12:477-483, 1984). We now report the results obtained following reverse-suturing experiments in which the initially deprived eye was reopened and the other eye occluded. The treatment with the internal ester of $GM_1$ (30 mg/kg/i.p. every 2 days) was performed only during the period of reverse-suturing. Ocular dominance distribution of area 17 neurons was assessed by conventional single unit recordings. A total of 519 cells in 6 control and 6 $GM_1$-treated kittens were studied. Results obtained indicate that in kittens treated with $GM_1$ the recovery of the initially deprived eye is less prominent than in control animals. Altough such data could appear contradictory with that previously obtained in monocularly deprived kittens, the shift of ocular dominance which occurs after reverse-suturing involves not only the recovery of inputs from the initially deprived eye but also the concurrent suppression of inputs from the newly deprived eye. As such, the diminished recovery of the originally deprived eye input observed with $GM_1$ treatment during the period of reverse-suturing may be due to enhanced maintenance of the newly deprived eye input. Therefore reverse-suturing data strengthen the hypothesis that $GM_1$ counteracts the effects of visual deprivation by supporting the maintenance of the connections from the deprived eye. Although the molecular mechanisms involved are still unknown, these findings are consistent with the hypothesis that gangliosides play a role in central nervous system (CNS) plasticity perhaps by regulating neuronotrophic interactions involved in the development and maintenance of CNS neuronal connections.

298.9 FACTORS INVOLVED IN AMBLYOPIA DEVELOPMENT IN EXPERIMENTALLY STRABISMIC MONKEYS. L.Kiorpes* M.Carlson*and R.Boothe. University of Washington, Seattle, Washington, 98195.

Two groups of monkeys (Macaca nemestrina) were made esotropic at ages ranging from 1 to 15 postnatal weeks. For one group, the esotropia was induced surgically. For the other group, the esotropia was induced by injection of Botulinum A neurotoxin into an extraocular muscle. The time course for the development of grating acuity was measured for each eye of each monkey.

Within both experimental groups, the monkeys could be divided into two subgroups: those who developed amblyopia (defined as an interocular acuity difference of greater than one octave) and those who did not. The two subgroups could be separated on the basis of fixation pattern. Basically, those who developed amblyopia chose to fixate primarily with the non-deviated eye, whereas those who did not develop amblyopia tended to alternate fixation. Such factors as size of deviation, refractive error, and age of intervention (esotropia induction) did not discriminate the two subgroups. One exception, however, was the animal whose esotropia was induced at 15 weeks. He showed primarily a monocular fixation pattern but never developed amblyopia.

Within the subgroup of surgically esotropic monkeys who developed amblyopia, there were consistent relationships between the extent of resultant amblyopia and both the age of intervention and the initial size of the esotropia. On the basis of data from the subgroup of neurotoxin induced strabismic monkeys with intervention ages of 4 weeks or less, the extent of amblyopia in this group depends on other factors.

298.10 EFFECT OF NEONATAL TRANSECTION OF THE THALAMIC RADIATIONS UPON THE DISTRIBUTION OF VISUAL CALLOSAL PROJECTION NEURONS IN THE GOLDEN HAMSTER. S.E. Fish, N.L. Chiaia and R.W. Rhoades. Neurobiology Program, Northeastern Ohio College of Medicine, Rootstown, OH 44272, and U.M.D.N.J.-S.O.M., Piscataway, NJ 08854.

Previous experiments (Cusick, C.G. and Lund, R.D., J. Comp. Neur., 212:385, 1982) have shown that thalamotomy in neonatal rodents alters the pattern of callosal terminals in visual cortex ipsilateral to the damaged diencephalon. However, the effect of disconnection of the cortex from thalamus upon the distribution of callosal projection neurons has never been examined. We have transected the thalamic radiations in newborn (<12 hrs. old) hamsters and used retrograde transport of horseradish peroxidase (HRP) to examine the distributions of visual callosal neurons ipsilateral and contralateral to the neonatal lesion when the animals reached at least 30 days of age.

The thalamic radiation lesions were incomplete in most animals, but they all resulted in extensive retrograde degeneration in the ipsilateral thalamus. In most cases, the dorsal lateral geniculate and lateral posterior nuclei were almost completely absent. The neonatal lesions also resulted in a consistent change in the distribution of callosal projection neurons in the cortex of the damaged hemisphere. On this side, HRP labelled cells were distributed in a nearly continuous band in layer VI and the lowermost part of lamina V across the entire mediolateral extent of areas 17 and 18a. The number of labelled neurons in the supragranular layers in the lateral part of area 17 and the medial portion of area 18a was, on the other hand, sharply reduced, relative to that observed in normal hamsters with similar HRP injections. In some neonatally brain damaged animals, no HRP labelled supragranular neurons at all were visible in this region. The effect of the neonatal lesion in this cortex thus appeared quite specific: A pronounced reduction in the number of supragranular, and virtually no effect upon infragranular callosal neurons.

The effect of the neonatal lesion in the intact hemisphere was much different. On this side, the number of labelled cells appeared abnormally low in most cases, but the tangential and laminar distribution of these neurons was not significantly different from that observed in normal hamsters.

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299.1 QUANTITATIVE DATA ON THE REGIONAL DISTRIBUTION OF THE DOPAMINE INNERVATION IN ADULT RAT CEREBRAL CORTEX. B. Lemay*, G. Doucet and L. Descarries (SPON: H.H. Jasper). Centre de recherche en sciences neurologiques (Département de physiologie), Université de Montréal, Montréal, Qué., Canada H3C 3J7.

The topographic distribution of the dopamine (DA) innervation in rat cerebral cortex has been well documented by earlier biochemical, fluorescence- and immuno-histochemical studies. However, there is still only semi-quantitative information as to the number of these axon terminals (varicosities) within the various cortical areas. To obtain such data, we used an *in vitro* radioautographic approach based on the uptake of [$^3$H]DA in fresh, transversal, 200 µm-thick slices of the whole cerebral hemisphere (Doucet, G. and Descarries, L., *Soc. Neurosci. Abstr. 10*, 422, 1984). The slices were incubated for 15 min at 35°C with $10^{-6}$M [$^3$H]DA and 5 x $10^{-6}$M desipramine in the presence of $10^{-4}$M pargyline, and radioautographed as large 4 µm-thick Epon sections exposed for 15 days. With the aid of a camera lucida, the labeled axonal varicosities were mapped at 4 transverse levels representative of the major cytoarchitectonic divisions of adult rat cortex. The highest density of DA innervation (number of labeled varicosities per unit of surface) was that of the supragenual cingulate areas, where the labeled terminals predominated in layers II and III. Slightly lesser overall densities of DA innervation were found in the antero-median cingulate cortex, suprarhinal and perirhinal claustrocortex and prepiriform area, with terminals mostly located within the deep layers of these regions. The entorhinal cortex showed a DA innervation of medium density spreading into all its layers except layer I. In the precentral, parietal and temporal areas, there was a DA innervation of low density which was restricted to layer VI. The occipital cortex showed exceedingly scarce DA terminals again confined to layer VI. Counts done on serial semi-thin (0.5 µm) sections exposed for various periods of time (7-30 days) indicated that, after 15 days, a plateau had been reached in the number of detected labeled varicosities. Besides, similar examination of serial sections cut at different thicknesses (0.5 - 4 µm) indicated that, beyond a section depth of 1 µm, an increasing number of labeled terminals failed to record their image into the emulsion. A factor was therefore calculated from these latter counts in order to correct the values obtained in 4 µm-thick sections. Lastly, the average diameter of the labeled endings was measured in electron microscope radioautographs from the same material. Under such conditions of specific labeling and optimal visualization, simple stereological extrapolation of the data will provide truly quantitative estimates of the areal and laminar densities of cortical DA innervation expressed as number of axon terminals per mm$^3$ of tissue. (Supported by grant MT-3544 from the MRC of Canada).

299.2 RADIOAUTOGRAPHIC QUANTIFICATION OF THE DOPAMINE INNERVATION IN ADULT RAT NEOSTRIATUM. G. Doucet, S. Garcia* and L. Descarries. Centre de recherche en sciences neurologiques (Dép. de physiologie), Université de Montréal, Montréal, Qué., Canada H3C 3J7.

In the rat, the total number of dopamine (DA) varicosities in one entire neostriatum has been approximated to $10^9$ by means of the Falck and Hillarp histofluorescence method (Andén, N.-E. et al., *Acta Physiol. Scand. 67*, 306, 1966). Since then, various studies have suggested that the intrastriatal distribution of these nerve endings is heterogenous, with patches of higher density, the so-called subcallosal streak and DA islands, as well as a slight decreasing rostro-caudal gradient. To evaluate the actual number of DA axon terminals in the various parts of adult rat neostriatum, 200 µm-thick slices of whole cerebral hemisphere were incubated for 15 to 60 min at 35°C with $10^{-6}$M [$^3$H]DA and 5 x $10^{-6}$M desipramine in the presence of $10^{-4}$M pargyline, and processed for light microscope radioautography as large 4 µm-thick Epon sections exposed for 7-30 days. However, in these conditions which lead to integral labeling of DA terminals in areas of relatively low DA innervation, [$^3$H]DA failed to label the varicosities in the core of the striatal portions of the slices. In contrast, following injection of 6-hydroxydopamine into the substantia nigra, residual striatal DA varicosities could be labeled throughout the full thickness of the slices. To acquire data in normal rat, the slices were therefore incubated at lower temperature (15°C) so as to diminish the efficiency of the reuptake mechanism and thus allow the tracer to overcome the "barrier" raised by the multitude of neostriatal DA varicosities. At this temperature, all DA varicosities could indeed be labeled throughout the full thickness of the slices. Yet, within certain areas of neostriatum, the extremely high density of DA innervation now precluded their direct counting in 4 µm-thick sections. Radioautographs had to be prepared from thinner section (0.5 µm) and to be exposed for shorter periods of time, when only a fraction of the labeled terminals were detected. A correction factor was then determined from counts carried out after various exposure times in less densely innervated portions of the striatum. A preliminary estimate of the mean density of DA innervation at one transverse level (A-8500 µm in König and Klippel's atlas) gave a figure in the order of $10^7$ per mm$^3$ of tissue. This value is consistent with the estimate of Andén et al. when extrapolated to the entire volume of the striatum. At the same level, the dorsolateral DA islands and the subcallosal streak reached a density of 9 x $10^7$ per mm$^3$ compared to 5 x $10^7$ outside these patches. Such quantitated data should be particularly useful for the study of various experimental models of growth or reinnervation and for correlations with other measurable parameters of DA function in the striatum. (Supported by grant MT-3544 from the MRC of Canada).

299.3 SEROTONIN INNERVATION OF RAT NEOSTRIATUM: IMMUNOHISTOCHEMICAL AND RADIOAUTOGRAPHIC STUDY. J.J. Soghomonian, L. Descarries and G. Doucet. Centre de recherche en sciences neurologiques (Dép. de physiologie), Université de Montréal, Qué., Canada H3C 3J7.

There is only fragmentary data regarding the distributional and fine structural features of the serotonin (5-HT) innervation in adult rat neostriatum. To obtain further information, three morpho-chemical approaches were used: immunohistochemistry with an antibody against 5-HT (IMMUNO); light microscope radioautography of tissue slices incubated with [$^3$H]5-HT (RAG *in vitro*); electron microscope radioautography after intraventricular administration of [$^3$H]5-HT (RAG *in vivo*). IMMUNO on histological sections taken at regular intervals across the striatum stains a relatively loose, ramified axonal network spread throughout but unevenly distributed within the nucleus. In addition to a slight caudo-rostral decrease, the 5-HT arborizations are less tightly intermingled in the dorsal and latero-dorsal than in the ventral sectors of neostriatum (and subjacent "ventral striatum"). Moreover, they appear somewhat crowded around the traversing myelinated fascicles of the internal capsule, as if they had been pushed into this location during development. RAG following the uptake of [$^3$H]5-HT *in vitro* or *in vivo* visualizes mainly axonal varicosities (terminals) rather than intervaricose segments. After incubation of whole hemisphere slices with $10^{-6}$M [$^3$H]5-HT and $10^{-5}$M non-radioactive noradrenaline in the presence of $10^{-4}$M pargyline, all but only 5-HT varicosities can be detected (Doucet, G. and Descarries, L., *Soc. Neurosci. Abstr. 10*, 422, 1984). In the striatum, the topographic distribution of the [$^3$H]5-HT-labeled endings is roughly proportional to that of the 5-HT-immunoreactive fibers. For instance, the actual number of 5-HT varicosities is at least twice higher in the medio-ventral (1.5 x $10^6$ varicosities per mm$^3$ on a first approximation) than in the latero-dorsal neostriatum (0.6 x $10^6$). In electron microscope radioautographs, the neostriatal terminals labeled *in vivo* with [$^3$H]5-HT are mostly of small caliber (0.2 - 0.5 µm in diameter), often barely larger than the thin unmyelinated fibers bearing them. In single thin sections, a very low proportion (less than 10%) exhibit membranous synaptic differentiations. The few observed junctions are made on dendritic spines and they are generally asymmetrical. These preliminary results illustrate the respective and complementary value of each approach in revealing various aspects of the 5-HT innervation within a given brain region. IMMUNO visualizes the full extent of the axonal network, allowing analysis of its intra-regional distribution. RAG *in vitro* is uniquely suitable for quantifying the innervation density as number of axonal varicosities per mm$^3$ of tissue. RAG *in vivo* remains the method of choice to characterize the intrinsic and relational features of these terminals. (Supported by grant MT-3544 from the MRC of Canada).

299.4 DISTRIBUTION OF MONOAMINES IN MONKEY HIPPOCAMPUS. C. A. Kitt, L. C. Walker, R. G. Struble, D. L. Price, T. H. Joh§, B. H. Wainer†, D. T. O'Connor*†† and M. E. Molliver. The Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205; §Cornell Univ. Med. Col., New York, NY 10021; †Univ. Chicago, Chicago, IL 60637; ††Univ. Calif., San Diego Sch. Med., San Diego, CA 92093

The pattern of monoaminergic (MA) innervation of monkey hippocampus was investigated using polyclonal antibodies directed against tyrosine hydroxylase (TH), dopamine ß-hydroxylase (DBH), serotonin (5HT), and a monoclonal anti-choline acetyltransferase (ChAT) antibody. Staining patterns of TH and DBH were compared to each other and to 5HT and ChAT in order to determine contributions of dopaminergic, noradrenergic, serotonergic, and cholinergic systems to the MA innervation of monkey hippocampus. MA fibers appear to enter the hippocampus through four main routes: the perforant pathway; alvear fiber bundle; association (axial) pathway; and fimbria/fornix. DBH-immunostained fibers course primarily along the alvear path, while few DBH-positive processes are seen in other fiber tracts. TH-positive fibers course through both the alvear and perforant pathways; however, many TH-immunostained fibers are also seen in the axial path and within the fornix. The majority of 5HT-immunostained fibers were seen in the alvear and perforant paths; however, a dense collection of 5HT-positive fibers was present in the portion of the axial path located within CA1. ChAT-immunostained fibers enter the hippocampus primarily through the fimbria/fornix. Few fibers were seen in the alvear, perforant, and axial paths. Differences in MA fiber densities were observed in CA1-4 regions. Fibers of all four monoamines are present in the dentate gyrus with axons scattered throughout the molecular layer of the dentate gyrus; the granule cell layer receives only sparse MA input -- most fibers appear to pass through the granule cell layer en route to polymorphic cells of the hilus. A thin band of ChAT-positive fibers were seen in the infra- and supragranular layers of the dentate gyrus. In CA3, the stratum oriens contains the most ChAT-positive fibers; few ChAT-positive fibers terminate in the pyramidal and molecular cell layers. The pyramidal cell layer and stratum moleculare of CA3, however, receives a particularly dense series of DBH, TH, and 5HT inputs. Stratum oriens and radiatum of CA3 receive sparse DBH, TH, and 5HT inputs. CA1 contains an intricate pattern of MA innervation; DBH, TH, 5HT, and ChAT-immunostained fibers are present in all layers, with a predominance of MA input to pyramidal neurons. A dense cluster of 5HT fibers are seen in the strata lacunosum and moleculare of CA1, perhaps terminating on the apical dendrites of pyramidal neurons. Clearly, monkey hippocampus receives a very rich MA innervation with an overlap in distribution, suggesting that MA inputs may converge onto individual hippocampal sectors and/or neurons.

299.5 SEROTONIN- AND SUBSTANCE P-LIKE IMMUNOREACTIVITY IN THE INTERPEDUNCULAR NUCLEUS: POSTNATAL DEVELOPMENT AND EFFECTS OF NEONATAL FASCICULUS RETROFLEXUS LESIONS. G. A. Barr*, T. C. Eckenrode* and M. Murray. (SPON: M.E. Goldberger). Biopsychology Program, Hunter College, City University of New York and Dept. Anatomy, Medical College of Pennsylvania, Philadelphia, Pa. 19129.

The interpeduncular nucleus (IPN) is a midline nucleus with cytoarchitectonically distinct subnuclei (Hamill & Lenn, *J. Comp. Neurol.*, **222**: 396, 1984). The IPN contains a number of putative neurotransmitters that arise both from extrinsic and intrinsic sources. Important afferents are the fasciculi retroflexi (FR) which contain major substance P (SP) and acetylcholine projections to the IPN. Furthermore, SP and specified types of synapses show degeneration and reinnervation following FR lesions (Artymyshyn & Murray, *J. Comp. Neurol.*, **231**: 78, 1985; Lenn et al., *Neurosci.*, **9**: 383, 1983; Murray et al., *J. Comp. Neurol.*, **187**: 447, 1979). Because lesions in young animals often produce effects that are different from those of lesions in adult animals, we studied the effects of FR lesions in neonates on the immunohistochemical distribution of SP, which projects to the IPN via the FR, and on the distribution of serotonin (5-HT) which arises from other sources.

The distribution of the putative transmitters was examined using the unlabeled antibody (PAP) technique of Sternberger. The normal development of each was defined in Sprague-Dawley pups sacrificed at regular intervals from birth to 3 months of age. Pups were anesthesized and perfused intracardially with saline followed by 4% paraformaldehyde. FR lesions were performed stereotaxically in 3 day old pups using the Heller adaptor for infant rats. Lesioned pups and unlesioned controls were assayed at 19 -21 days of age and 40 - 42 days of age.

SP-like immunoreactivity developed in stages (Eckenrode et al., *Neurosci. Abst.*, **9**: 987, 1983). The SP projection to the lateral subnucleus was present at birth, but staining was absent from the other subnuclei. Adult distribution of immunoreactivity was seen at 17 days of age, with staining present in the rostral, central and dorsal subnuclei. In contrast, the distribution of 5-HT - like immunoreactivity in the infant was similar to that of the adult although with lower density. Staining was evident in the dorsal and rostral subnuclei and the caudal aspects of the lateral subnuclei.

At the shorter survival time following unilateral lesions, ipsilateral loss of SP was seen in the lateral aspects of the lateral subnuclei. The loss from the infant lesions was similar to that described for the adult. There was no noticeable change in the other subnuclei. The distribution of serotonin was similar to that seen in normal infants and adults.

Thus, the 5-HT system in the IPN is an early developing system. SP develops more slowly, appearing in the lateral subnuclei prior to the rostral, central or dorsal subnuclei. Lesions of the FR in infants produced a loss of SP similar to that seen following FR lesions in adults. There was no evidence of heterotypic sprouting by serotonin, at least at the short survival times used here. Supported by NIH NS16556.

299.6 MAO A IN CATECHOLAMINERGIC AND MAO B IN SEROTONERGIC REGIONS: IMMUNOCYTOCHEMICAL LOCALIZATION IN HUMAN BRAIN. K.N. Westlund, R.M. Denney*, R.M. Rose, and C.W. Abell.* Depts. of Human Biol. Chem. & Genetics and Psychiatry & Behav. Sciences. University of Texas Medical Branch, Galveston, TX 77550.

In human autopsy brain, two distinct populations of MAO-positive neurons which corresponded to known monoaminergic cell groups were identified with two highly specific monoclonal antibodies (MAO A-7E10 and MAO B-1C2) elicited to human placental MAO A and human platelet MAO B. One cm thick slabs of human brain were immerse-fixed in 10% buffered formalin for 5-30 days prior to overnight equilibration in 20% buffered sucrose. Frozen 30-40 μm sections were cut on a sliding microtome, and the tissues collected and rinsed in phosphate buffer. Overnight incubation in 1:1,000 dilution of ascites fluid preceded localization by standard PAP immunocytochemistry. Immunocytochemical controls included omission of one or more of the reagents in the PAP protocol, dilution of the antibody, competition of the antibody with antigen, and incubations with ascites fluid from mice injected with vehicle only. Specific staining was not seen in control stains or when the antibody was preadsorbed with antigen (10 μg/ml).

MAO A was localized in regions containing catecholamine neurons designated cell groups A1-A14 by Dahlström and Fuxe (1964), including known noradrenergic cell groups such as the nucleus locus coeruleus and cells scattered in the commissural and solitary nuclei, and known dopaminergic cell groups such as the substantia nigra and the periventricular nucleus of the hypothalamus. Under the conditions used, the number of cells containing detectable levels of stain for MAO A in the substantia nigra did not represent all dopaminergic cells normally observed in this region in other species. MAO B was localized in regions containing serotonergic neurons designated cell groups B1-B9, including the raphe complex and the nucleus centralis superior. Furthermore, MAO A and MAO B staining were observed in the scattered magnocellular components of the hypothalamus, in varicose terminations, and in some astrocytes throughout the neuraxis. Both forms of the enzyme were localized in distinct but intermingled populations of neurons in the hypothalamus. Similar or identical localization of MAO A and B has been observed in monkey brain, using similar methods. The clear segregation of MAO A in catecholamine regions and MAO B in serotonergic regions suggests that these enzymes perform different physiological roles. (Supported by PHS grants MH34757, NS19543, NS07309, and the Multidisciplinary Research Program on Schizophrenia, University of Texas Medical Branch, Galveston TX.)

299.7 MORPHOLOGY AND SYNAPTIC RELATIONSHIPS OF NORMAL AND SEROTONERGIC PROCESSES IN CAT RAPHE DORSALIS: A FINE STRUCTURAL SERIAL ANALYSIS. G. Chazal* and H.J. Ralston III. (SPON: C. Morgan) Dept. Anatomy, Univ. California School of Medicine, San Francisco, CA 94143.

Most of our present knowledge of the ultrastructure of serotonergic (5-HT) neurons in the nucleus raphe dorsalis (RD) is derived from autoradiographic studies using radiolabeled 5-HT on rats. There have been relatively few studies in the cat and none has given a detailed morphological description of the 5-HT intrinsic innervation. It is to this purpose that we undertook a study using an antibody to 5-HT to describe the serotonergic profiles in cat RD and to determine, by analysis of serial thin sections, their synaptic relationships. In addition, a comparison with the ultrastructural features of RD profiles in conventional, aldehyde-fixed material, was conducted to clarify the nature of the structure, i.e. dendrite or axon terminals, of some 5-HT processes.

For immunocytochemical (ICC) procedures, adult cats were anaesthetized and then perfused with 4% paraformaldehyde-0.1% glutaraldehyde. The brain stem was removed and sectioned on a vibratome at a thickness of 30 or 50 um. Tissue sections were incubated for 12 hrs in 5-HT antisera (1/400-1/800 dilution) and then processed by the PAP method. Following osmication, dehydration and epon embedding, sections from the RD were cut in serial 4 um sections and areas were subsequently selected, for serial ultrathin sectioning. Brain stem tissue from other cats was prepared following perfusion fixation with 2% paraformaldehyde-2% glutaraldehyde and subsequent osmication, embedding and staining.

In the tissue processed using the ICC method, we observed different types of 5-HT profiles. Large dendritic shafts (>1.5μm diameter) which contained PAP reaction product comprised the dominant population. In addition, several immunoreactive axonal varicosities were also present and easily distinguished by the presence of electron-dense PAP reaction product that coated the outer membranes of vesicles. Some of these varicosities were observed to participate in synaptic junctions. Another type of process appeared to be dendritic by the usual criteria of content but also exhibited groups of vesicles. In raphe dorsalis sections prepared for conventional EM, we frequently observed dendritic profiles containing vesicles, some of which established synaptic contacts with other dendrites and thus could be interpreted as presynaptic dendrites (PSD). Others presented clusters of vesicles accompanied by unusual dense structures larger than the vesicles themselves. These results suggest that some 5-HT profiles containing vesicles are dendrites. These data are consistent with the hypothesis that a local serotonergic system, including dendritic release of 5-HT, regulates the activity of raphe dorsalis neurons.

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299.8 IMMUNOHISTOCHEMICAL LOCALIZATION OF SEROTONIN NEURONS IN AUTONOMIC REGIONS OF RAT SPINAL CORD. B.W. Newton & R.W. Hamill, Department of Neurology, University of Rochester/Monroe Community Hospital, Rochester, New York 14603

5HT is one of the neurotransmitters implicated in the control of autonomic functions, e.g. blood pressure and sympathoadrenal function. Spinal 5HT is thought to originate from descending projections from brainstem raphe neurons synapsing in spinal preganglionic areas. Although 5HT spinal neurons have been described in other species (monkey, stingray), previous studies have not revealed these neurons in rat. This is the first known report of 5HT neurons in the rat spinal cord, and describes the existence of 5HT neurons in spinal autonomic regions.

In order to study the distribution of serotonin-like immunoreactive (5HT-LI) neurons in rat spinal cord, 21 adult female rats were examined with the PAP method. Ten or 30 days prior to perfusion spinal cords of 4 rats were transected at the T4-T5 junction. One hr before perfusion 8 rats were pretreated with L-tryptophan and a MAO inhibitor, either pargyline or tranylcypromine. Rats were perfused with Zamboni's fixative and the spinal cords were serially sectioned at 40um on a vibratome. Sections were incubated for 48 hrs in antiserum generated against 5HT (R21M) coupled to bovine serum albumin (BSA; technique of Maley & Elde, Neurosci., 7:2469, 1982) diluted 1:5000. All antibodies were diluted in phosphate buffered saline/3% normal sheep serum with 0.3% Triton X-100. Absorption controls (5HT, BSA, tryptophan, tryptamine, epinephrine, norepinephrine, dopamine) demonstrated R21M specificity towards 5HT.

5HT-LI soma were observed in normal rats, although a greater number of 5HT-LI neurons were found in pretreated rats. 5HT-LI neurons were found in spinal cord regions known to contain preganglionic autonomic neurons. The majority of these 5HT-LI neurons were located in the dorsal commissural nucleus and just lateral to the central canal. The most rostral and caudal 5HT-LI neurons were at T3 and S4, but the greatest number occurred between levels T7-T10. Most 5HT-LI neurons appeared as bipolar fusiform cells oriented longitudinally to the long axis of the cord. However, multipolar cells were observed. 5HT-LI neurons were found among a network of 5HT-LI fibers resembling previously described enkephalinergic patterns. In rats with spinal cord transections 80-90% of the 5HT-LI fibers disappeared below transection, but 5HT-LI soma were still observed. These 5HT-LI neurons are the probable source of the remaining 5HT-LI fibers below the transection and may also explain the origin of intrinsic 5HT, eluciated biochemically, below spinal cord transections. 5HT spinal neurons may serve as interneurons participating in the serotoninergic control of preganglionic autonomic neurons. Supported by Univ. of Rochester/Monroe Community Hosp. Research Fund & Neurobiology of Aging Training Grant AGT32107.

299.9 ULTRASTRUCTURAL HISTOCHEMISTRY OF BIOGENIC AMINE LOCALIZATION IN INTERNEURONS OF THE CARDIAC GANGLION IN NECTURUS MACULOSUS. R.M. Kriebel, A. Angel*, D.S. Neel*, and R.L. Parsons. Dept. of Anatomy & Neurobiology, University of Vermont College of Medicine, Burlington, VT 05405.

The parasympathetic cardiac ganglion in the mudpuppy, N. maculosus, contains postganglionic principal cells and interneurons. Several bioactive substances have been localized in the interneurons which may have integrative effects at synapses within the ganglion. Ganglionic interneurons can be identified electron microscopically by the presence of numerous cytoplasmic granules 80 - 120 nm in diameter. Throughout the ganglion there are bundles of unmyelinated fibers some of which are filled with granular and agranular vesicles and axosomatic terminals with similar vesicles synapsing on principal cells. Structural features alone are not sufficient to determine the chemical basis of synaptic circuitry within a system. Therefore, to understand the aminergic contribution to ganglionic synaptic circuitry the chromaffin reaction was used. Ganglia were immersion fixed in 2% para- and 2% glutaraldehyde in sodium chromate/potassium dichromate buffer (pH 7.2) for 30 min at 4°C, incubated in chromate buffer at pH 6.0 for 18 h, osmicated, dehydrated, and embedded in epon. Thin sections were stained with lead citrate to visualize the chromaffin reaction product and studied in a JEOL 100CX II STEM. In these preparations, interneurons were readily identified by their characteristic intracellular granule population. All interneurons identified showed granules which were positively labelled by the chromaffin reaction. In each interneuron, there appears to be an electron dense heterogeneity of reaction product in the granules. Many granules are intensely electron dense while others cannot be differentiated from background tissue density. This heterogeneous appearance may reflect different levels of amine content or the presence of granules which contain other substances. Granules within unmyelinated fibers were also labelled. Dense cored granules in synaptic terminals on principal cells were also labelled indicating an aminergic synaptic innervation to these cells. In confirmation of previous studies, the interneuron cell bodies, ensheathed with supportive glial-like cellular processes, rarely receive synapses. Elemental microanalysis was used to verify the chromium content of the reaction product within the dense cored granules. Studies are in progress to further define the interaction of interneuronal synapses with other interneurons and the principal cells of the cardiac ganglion.

299.10 NEW HISTOCHEMICAL STAIN FOR HISTAMINE. A. Li* and R.J.Dinerstein University of Chicago, Dept. Pharmacol. & Physiol. Sci., Chicago, IL 60637 and Merrell Dow Research Institute, Indianapolis, IN 46268-0470.

Histamine (HA) is a putative neurotransmitter in the mammalian brain and is known to be a neurotransmitter in Aplysia. We have developed a simple fluorescent technique that is specific for histamine.

Histamine is known to undergo a condensation reaction with vitamin B6 under physiological conditions to form a tetrahydroimidazopyridine (TIP). The reaction product formed between HA and vitamin B6 is different from the normal Schiff product formed between other amines and vitamin B6. Based on this observation, a number of vitamin B6 analogues were examined and salicyladehyde (SA) was found to form a similar derivative with HA in MeOH/$H_2O$ at pH10. In a polyvinylpyrrolidone (PVP) matrix, the TIP derivative of HA exhibits a green fluorescence (ex max: 375 nm; em max: 495 nm) that increases in intensity and changes to a yellow fluorescence (ex max: 420 nm; em max 530 nm) after exposure to UV light. The chemical basis for this change is a photochemical singlet oxygen reaction. This fluorescence is distinctly different from that seen for other similarly treated biogenic amines and amino acids. Rat mast cells isolated from peritoneal washes were treated with SA at pH 11. The resulting cellular fluorescence was found by microspectrofluometry to be identical to that for the TIP derivative of HA. In stretch preparation of rat peritoneum, alkaline SA produced the same fluorescence in mast cells. Furthermore the intensity of the background mesentary fluorescence was less than 15% of the mast cell fluorescence. Using mast cells as a model we have developed a specific histochemical method for histamine which could be used in studying histamine in neurons. This research supported by Mental Health Training Grant 1 T32 MH 14274-09 and NIH GM-22220, Brain Research Foundation, University of Chicago.

299.11 WHEATGERM AGGLUTININ APO-HRP-GOLD: A NEW TRACER FOR LIGHT AND ELECTRON MICROSCOPY. Basbaum, A. I. and Menetrey, D*. Dept. of Anatomy, University of California San Francisco, CA 94143 and INSERM U-161, 75014 Paris, France.

There is great interest in the development of anatomical techniques for the study of axon collateralization in the CNS. The introduction of transportable dyes has significantly simplified such studies. For EM analysis, however, electron dense markers are required. To this end, Menetrey and Lee ('85) have recently described a technique that couples colloidal gold to a wheatgerm agglutinin (WGA)-HRP conjugate. The resultant molecule is a very sensitive tracer that can be used for retrograde transport studies at both the LM and EM level. Double labelling studies with immunocytochemistry are also possible, however, in that case, the HRP in the gold complex must first be inactivated so that there is no interference with detection of, for example, the PAP molecule. Unfortunately, this latter treatment is harsh and thus the preservation of the tissue for EM analysis is reduced.

To overcome the problem, we have adapted the approach of Hayes and Rustioni ('79) and in collaboration with Sigma have conjugated WGA to the enzymatically inactive apoprotein (Apo-HRP). Although the gold coupling properties of the WGA-Apo-HRP conjugate differ somewhat from that of WGA-HRP, LM studies established that WGA-Apo-HRP-gold is also a very sensitive retrograde tracer. Somatic and dendritic labelling of retrogradely labelled neurons is readily detected after silver enhancement of the gold particles. We have also sucessfully used the new complex in combined immunocytochemical studies. We are presently evaluating its properties at the EM level, (both with and without silver enhancement), in combination with immunocytochemistry as well as with the anterograde and/or retrograde transport of enzymatically detected, active WGA-HRP. The possible use of WGA-Apo-HRP-gold in anterograde studies is also being assessed.

By coupling different size gold particles to the new complex, we believe that multiple retrograde tracers can be generated. Finally, the complex should prove of value in studies of the cell biological mechanisms underlying axonal transport.

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299.12 BENZIDINE DIHYDROCHLORIDE (BDHC) AS A CHROMOGEN FOR LIGHT AND ELECTRON MICROSCOPIC IMMUNOCYTOCHEMISTRY. S. Lakos* and A. I. Basbaum. (SPON. J. LaVail) Dept. of Anatomy, University of California San Francisco, CA 94143

Despite the fact that there are very sensitive chromogens available for the localization of horseradish peroxidase (HRP), these have rarely been used to detect the PAP molecule in immunocytochemical studies. Often this is because the reaction product is soluble and thus difficult to preserve for EM analysis. Peschanski and Ralston ('85), however, recently adapted the chromogen, BDHC, for anterograde and retrograde studies. In this report, we describe its use for immunocytochemistry.

The protocol is similar to that typically used with tetramethylbenzidine (Mesulam, '78), with several modifications. Although the greatest sensitivity is produced at low pH (e.g. 3.5, like TMB), to preserve ultrastructural quality, the BDHC reaction is run at pH 6.5. This results in a reaction that is still considerably more sensitive than DAB. There is not only much more reaction product, (at higher dilutions of primary antisera, e.g. against 5HT), but perfusion fixatives can also contain up to 1% glutaraldehyde. The increased sensitivity is also reflected in the background staining, however, treatment of sections in low glutaraldehyde after the PAP step can differentially increase signal. The BDHC reaction product is particulate and has a blue-green color that is very different from the reddish-brown DAB reaction product. We have used these color differences for LM double labelling studies.

To preserve the BDHC product for EM analysis, osmication must be carried out in an s-collidine buffer at 45°C. Dehydration and embedding are standard. At the EM level, the BDHC reaction product consists of discrete, electron dense crystals that contain parallel cross bridges. Since the crystals are readily distinguished from the flocculent DAB reaction product, EM double labelling studies are possible. To this end, we have detected retrogradely transported WGA-HRP with DAB and combined this with the EM immunocytochemical localization of 5HT in the medullary raphe.

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300.1 THE MODELING OF HIPPOCAMPAL CONVULSANT ACTIVITY USING AMPLITUDE AND FREQUENCY DAMPED SINE WAVES. L.W. Jennings* and H.J. Doller*. (SPON: G. Drust) Depts. of Internal Medicine and Neurology, UTHSCD and Epilepsy Center, VAMC, Dallas, TX.

The rhythmic discharges recorded from the in vitro hippocampal slice exposed to picrotoxin are fit to one or more amplitude and frequency damped sine waves. Such analysis allows the response to be described by as few as 5 parameters: delay to start of response (T), amplitude (A), amplitude decay (B), frequency, and frequency decay (g(t)). The general formula is:

$$y(t) = a + bt + A \exp[-Bt] \sin[\, g(t)\,(t-T)] + e(t)$$

where a+bt is a linear base line, g(t) is a function of time chosen to describe the change in frequency observed with time, and e(t) is the residual error term.

Rat hippocampi were quickly removed, sliced (450 uM), and placed in a trough-like chamber. Stimulating electrodes were placed in the perforant pathway and a glass recording electrode (1 to 2 M ohms) in the CA1 cell body layer. After a recovery period, single stimuli evoked a field containing a single population spike (Doller & Weight, Brain Res. 237:1, 1982). When picrotoxin (0.1 mM) was added to the superfusate, multiple population spikes (3 to 7) were evoked by a single stimulus.

The data were fit to an amplitude and frequency damped sine wave using the following strategy. Ordinary least squares techniques were used to estimate the linear trend, a+bt. Using the residuals, z=y-(a+bt), the remaining parameters were estimated using the Marquardt method for nonlinear regression by least squares. Initial estimates for A and B were obtained by fitting ln(z)=ln(A)+Bt to the absolute values of the extremes of the curve. The form and initial estimates for g(t) were obtained by analyzing the frequency estimates f=1/Δt, where the t's are the locations of the extremes.

This damped sine wave model may be useful in quantifying seizure activity and the actions of convulsant and anticonvulsant drugs. In addition Fredman (Ann. Rev. Biophys. Bioeng. 1:225, 1972) suggested that in the olfactory cortex cells the normal rate of neuronal firing resembles an amplitude damped sinusoid. O'Connor (EEG, 59:497, 1984) has recently used a summation of amplitude damped sine waves to model clinically evoked visual potentials. The hypothesis is that the general neuronal circuitry pattern of feed back excitation and inhibition leads to the production of damped sine wave potentials. Evoked potentials are the summation of different waves produced by specific groups of neurons. Changes in a parameter of such a model may be related to a specific change in a group of neurons. The current investigation suggests that the more general model for fitting evoked potentials may be the summation of amplitude and frequency damped sine waves.

300.2 MULTINEURON ANALYSIS REVEALS COMPLEX PATTERNS OF INTERACTION AMONG NEURONS IN FOREBRAIN NETWORKS AND CARDIORESPIRATORY PARAMETERS DURING SLEEP-WAKING STATES. R.D. Frostig, Z. Frostig*, R.C. Frysinger, V.L. Schechtman, and R.M. Harper. Neuroscience Program, Brain Research Institute, and Department of Anatomy, UCLA, Los Angeles, CA 90024.

Several studies demonstrate the existence of forebrain areas participating in the control of the cardiovascular and respiratory systems. Part of this evidence derives from cross-correlation studies between forebrain units and cardiorespiratory parameters. Each cross correlation, however, can detect interactions between only a single neuron and a single cardiorespiratory parameter. The study of simultaneous interactions among neurons and cardiorespiratory parameters may reveal the existence of unique interactions that would not be detected by examining only pairs of interactions.

We studied local networks of four or five simultaneously recorded single units in forebrain areas that participate in cardiovascular and respiratory control, the central nucleus of the amygdala and the medial frontal cortex. Neuronal and cardiorespiratory measures were recorded in freely moving, drug-free cats during different sleep-waking states, so that an experimental manipulation (sleep states) could induce potential alterations in their interactions.

We used the joint multicorrelation procedure (Frostig, R.D., et al., Soc. Neurosci. Abs., 10: 756, 1984) to analyze interactions among neurons in these networks and cardiorespiratory parameters that could be represented as point processes (e.g., the QRS peak in the ECG, the onset of respiration). Analysis of these neuronal and cardiorespiratory processes (up to seven simultaneously) revealed the existence of complex recurring patterns of interaction among neuronal discharges and among neuronal discharges and cardiorespiratory activity. These patterns consisted of repetitions of spike discharge from different spike and cardiorespiratory trains (e.g., AB interval of 4 ms, followed by BC interval of 9 ms, followed by CD interval of 10 ms, etc.). The pattern's recurrence was statistically significant and involved up to five simultaneous processes appearing with no, or minimal, variation in their timings (1- and 5-ms bins, respectively). Many patterns could appear at the same recording session, and changes in sleep-waking state produced different patterns.

These findings suggest the presence of complex coding properties involving many neurons in networks within forebrain areas and cardiorespiratory parameters. These interrelations are altered by changes in sleep and waking state.

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300.3 DOES GLYCINE INHIBITION MEDIATE AIRSTEPPING AND PAW-SHAKE RESPONSES IN SPINAL CATS? C.A. Giuliani, S.H. Chandler, G. Caneta*, and J.L. Smith. Dept. of Kinesiology, UCLA, CA, 90024.

Rhythmical hindlimb movements, including airstepping (ASTP) and paw-shake response (PSR), are thought to be centrally generated by the lumbosacral spinal cord. However, neural mechanisms of the central pattern generator (CPG) controlling these rhythmical behaviors is unknown. Recent evidence suggests that glycine may mediate the inhibitory coupling that controls alternating segmental activity during lamprey swimming (Grillner and Wallen, Acta Physiol Scand. 110:103, 1980; Cohen and Harris-Warrick, Brain Res. 293:164, 1984). The purpose of this study was to investigate the role of glycine inhibiton mediating intra- and interlimb control during ASTP and PSR in cats spinalized at T-12.

Bipolar electrode wires were implanted in selected flexor and extensor muscles of the hindlimbs. A cannula (PE 10) was implanted in the subarachnoid space at the $L_4$ vertebral level and affixed to the skull (adapted from Yaksh and Rudy, Physiol Behav., 17:1031, 1976). The effects of strychnine (a glycine antagonist) on the intralimb synergies of ASTP and PSR and the interlimb coordination of ASTP were assessed by EMG and videotape records before and after injection of strychnine and control injections of the vehicle solution (saline).

High doses of strychnine (>50 ug/200 ul) produced seizure-like movements, characterized by cocontraction of all muscles recorded for 2-10 minutes following injection. During this period no ASTP was observed, however, PSRs were easily elicited. Recovery to control behavior returned within an hour. During recovery or following intermediate range doses of strychnine, interlimb coordination of ASTP was severely affected. Variability in the phase relationships between limbs occurred frequently. Additionally, independent hindlimb activity was noted when one hindlimb airstepped and the other was silent or exhibited cocontraction of antagonists. Neither the average cycle period, nor the intralimb synergy of ASTP appeared affected; however, the integrated EMG doubled. Intermediate doses of strychnine tended to increase the cycle period of the PSR, but did not disrupt the characteristic intralimb synergy. Low doses of strychnine (<10 ug/200ul) had no apparent affect on ASTP or PSR.

Our data suggest that glycine inhibition may be more important in mediating interlimb than intralimb coordination of ASTP in the spinal cat. Furthermore, our results indicate that while glycine mediated synapses may be important in the production of ASTP, they do not appear to be as important for the production of the PSR. Supported by NIH grant NS 19846.

300.4 PHARMACOLOGICAL ACTIVATION OF LOCOMOTOR ACTIVITY IN THE DEVELOPING SPINAL CORD OF THE CHICK EMBRYO. M. Barry* and M.J. O'Donovan. (Spon: I. Bruce) Dept. of Physiology and Biophysics, Univ. of Iowa, Iowa City, IA 52242.

The purpose of this study was to analyze the role of excitatory amino acids in the initiation of embryonic motor activity. Locomotor activity was recorded from antagonistic hindlimb muscles or muscle nerves in St.32-36 chick embryos using a superfused isolated spinal cord preparation (Landmesser and O'Donovan 1984; J. Physiol., 347:189, 1984). We found that bath applied NMA (N-methyl-DL-aspartate was effective in influencing motor activity over a narrow range of concentrations (3-30μM). Low doses of NMA initiated spontaneous burst activity in quiescent preparations and increased the frequency of otherwise normal burst episodes in spontaneously active embryos. Higher concentrations (30μM) of NMA activated continuous bursting in which the pattern of muscle activity was similar to that observed during spontaneous activity (see Fig. 1). At concentrations >50μM flexor and extensor motoneurons were tonically activated and rhythmic activity was abolished. In younger embryos (St.32), in which the pattern of antagonist alternation was not yet mature, NMA increased the frequency of burst episodes but did not result in the appearance of alternating activity. Application of the NMA antagonist 2-amino-5-phosphonovalerate (2-APV) did not block spontaneous motor activity suggesting that non-NMDA amino acid receptors may be involved in the generation of spontaneous motor activity in the chick embryo.

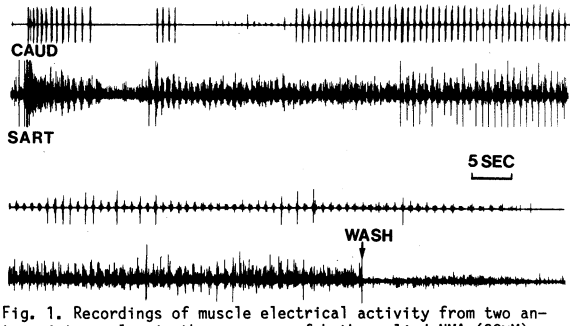


Fig. 1. Recordings of muscle electrical activity from two antagonist muscles in the presence of bath applied NMA (30μM). Lower set of records is after 45 mins of continuous activity. (Supported by NSF BNS-02838).

300.5 SEGMENTAL PROPERTIES OF THREE FORMS OF SCRATCH REFLEX IN THE SPINAL TURTLE: LOCALIZATION OF INPUT DERMATOMES AND CENTRAL PATTERN GENERATING ELEMENTS. L.I. Mortin and P.S.G. Stein, Biology Dept., Washington Univ., St. Louis, MO 63130.

The spinal turtle, immobilized via neuromuscular blockade, produces three distinct forms of the scratch reflex (J. Neurophysiol. 53:1533, 1985). We have investigated the organization of 1) the afferent inflow which initiates each scratch form, and 2) the localization of central pattern generator (CPG) elements within various spinal cord segments. The spinal cord of Pseudemys scripta elegans consists of the following segments: 8 cervical segments; 10 dorsal segments (D1-D10, equivalent to the mammalian thoracic and lumbar segments); 2 sacral segments (S1-S2); and about 16 caudal segments (Ca1-Ca16). All turtles are spinalized between D2 and D3, just posterior to the forelimb enlargement. The hindlimb enlargement consists of D8-D10, S1 and S2.

The rostral scratch receptive field is innervated by afferents entering D3-D6; the pocket scratch receptive field is innervated by D6-D8; the caudal scratch receptive field is innervated by Ca1, other caudal segments, and sometimes S2. There is a striking discontinuity in the innervation of the anterior part of the anal shield, ventral to the hip: spinal segments D8 and Ca1 innervate adjacent and overlapping regions of the shell and skin. Segments D8-S2 innervate the hindlimb skin.

The motor pattern for each scratch form consists of at least three phases of activity per cycle. Each scratch form can be produced if the five segments of the hindlimb enlargement are connected with a segment whose dermatome innervates some of the receptive field for that scratch. Removal of additional segments may affect parts of each scratch form's CPG. A rostral scratch is produced with D8-D10 connected to segments innervating the rostral receptive field; the posterior 40% of the enlargement is not needed for rostral scratch production. After removal of D10, leaving D8-D9 connected to more anterior segments, rostral scratches are produced with little or no hip retractor phase, but otherwise normal motor patterns. A pocket scratch motor pattern is produced with D7-D9 or D8-D10. D8-D9 alone produces a pocket rhythmicity with missing elements. These results imply that the pocket scratch CPG is distributed over the four segments D7-D10. A largely normal caudal scratch can be generated by the enlargement connected to more caudal segments. Removal of D8 reduces or eliminates the hip protractor phase of the caudal scratch motor pattern; further experiments will clarify the contributions of different segments to the caudal scratch. The entire enlargement is not necessary for the production of every scratch form. As few as 3 spinal cord segments can produce a normal scratch pattern. From these experiments we will determine the segmental localization of shared and unique elements of the CPG circuitry for each scratch form.

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300.6 MOTOR PATTERNS EVOKED BY STIMULATION OF THE OPTIC TECTUM IN TWO SPECIES OF WEAKLY ELECTRIC FISH. J. Yuthas* (SPON:H. D. Christensen). Dept of Zoology, University of Oklahoma, Norman, OK 73019.

Previous studies have shown that electrical stimulation of the optic tectum results in orientation, escape or avoidance behaviors in toads (reviewed in Ewert, Burghagen and Schurg-Pfeiffer, Advances in Neuroethology 56A) and iguanas (Stein and Gaither, J. Comp. Neurol. 202:69).

Both the glass knife (Eigenmannia virescens) and the brown ghost (Apteronotus leptorhynchus) have relatively simple locomotor patterns. They propel themselves by generating sinusoidal waves along their anal fin. The direction of wave propagation can be from head to tail (forward swimming) or from tail to head (backward swimming). To quantify the swimming responses to tectal stimulation I glued the exposed skulls of anesthetized fish to a two-axis strain gauge. Responses to stimulation were also recorded on videotape. Fish responded to 0.5 sec trains of stimulus pulses at rates of 20 to 100Hz and amplitudes of 25-100uA with anal fin reversals or accelerations, body flexions and pectoral fin movements. No eye movements were observed. Stimulation in the rostral tectum caused backward swimming (reversal, negative forces). Stimulation in the caudal tectum caused an acceleration of forward swimming (positive forces). Constant intensity stimulations at several points along the caudal to rostral axis (the caudal-most point of the tectum was designated '0' the rostral-most was '100') showed that the response of the anal fin to stimulation changes from acceleration to reversal at 60 in Apteronotus and 66 in Eigenmannia. The largest positive forces (>0.7grams/gram fish) were found between 20 and 40. The largest negative forces (-0.5grams/gram fish) were found at about 70 in Apteronotus and at 85 in Eigenmannia.

In Apteronotus stimulation in the left tectum generally caused a body flexion that brought the tip of the fish's tail to the left of its longitudinal body axis. This response seemed to be independent of body position at the start of stimulation. As the stimulation site progressed from rostral to caudal the final position of the tip of the tail moved further anterior due to a decreasing radius of curvature of the bend. A mid-body flexion to the right often complicated the leftward tail flexion. Stimulation in the rostro-lateral tectum caused rigid braking movements of the pectoral fins. The pectoral fins can respond independently of each other and at more caudal stimulation sites complex rhythmic movements were evoked. The responses evoked by stimulation are site specific and appear to be avoidance responses. Supported by NIH grant #NS12337 to J. Bastian.

300.7 A MODEL OF PATTERN GENERATION OF INSECT WALKING RECONSIDERED. S.N. Zill, Dept. Anatomy, Univ. Colo. Med. Sch., Denver, CO 80262

Cockroaches that have been decapitated or that have cut thoracic connectives can show rhythmic bursting in motoneurons to intrinsic leg muscles. These preparations have been studied as models of walking (Pearson, Sci. Am., 235:72, 1976) and to evaluate the functions of leg proprioceptive sense organs. Several subsequent studies have, however, raised doubts as to whether motoneuron bursting in headless cockroaches actually reflects attempts at walking or other behaviors such as righting responses (Reingold and Camhi, J. Insect Physiol. 23:1407,1977) when an animal on it's back tries to turn upright. The present study was undertaken to 1) examine which behavior is associated with bursting in these preparations and 2) to determine the usefulness of this preparation in evaluating the functions of sensory inputs in walking.

Myographic recordings were taken conventionally from four muscles, the extensors and flexors of the trochanter and tibia, in the hindlegs of freely moving cockroaches. Animals were first released into an arena and motor activity in walking was recorded. To record righting responses animals were either picked up and placed on their backs or tripped while running. The same behaviors were re-examined after decapitation and cutting of thoracic connectives.

Intact cockroaches showed rhythmic activity in leg motoneurons during both walking and righting. However, the phase relations of bursting in synergist muscles of the two joints differed in each of these behaviors. In walking, bursting was tightly coupled in synergist muscles while phase relations were more variable in righting.

After decapitation or cutting of thoracic connectives, cockroaches walked extremely poorly and slowly (mean rate, 2.5 cm/hr + 1.4 SD) with severe discoordination of motoneuron bursting and extensive co-contraction of antagonist muscles. All animals, however, showed rhythmic bursting when placed on their backs, which resembled righting responses of intact preparations in phase relations of synergist muscles. Rhythmic activity was completely inhibited when animals were subsequently turned upright and had sensory inputs normally occurring in posture and locomotion.

It is therefore concluded that motoneuron bursting in these preparations does not represent attempts at walking but righting responses. Headless cockroaches may still be useful preparations for studying the motor mechanisms underlying both these behaviors but are not of value in testing the functions of leg sense organs.

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300.8 HOMOLOGOUS PYLORIC MUSCLES ARE INNERVATED BY DIFFERENT MOTOR NEURONS THAT USE DIFFERENT NEUROTRANSMITTERS IN PANULIRUS AND CANCER. M.B. O'Neil*, S.L. Hooper, R.J. Wagner*, & E. Marder. Biol., Brandeis Univ., Waltham, MA 02254.

The motorneurons of the pyloric system of the stomatogastric ganglion of decapod crustaceans function both to excite the muscles of the animal's stomach and to participate in the generation of the foregut motor patterns. These neurons were named and identified on the basis of which muscles they innervate (Maynard & Dando, Phil Trans Roy Soc Lond B, 1974). In Panulirus interruptus there are two PD neurons that innervate two sets of extrinsic muscles (one attachment on an ossicle of the stomach; the other on the hyperdermis). Although the pyloric rhythm in the crab, Cancer borealis, is almost indistinguishable from that of Panulirus, there are four neurons in the crab that fire together and were called PD neurons (Hermann, J. Comp. Physiol., 1979). We now find that these 4 PD neurons fall into two classes that innervate different muscles. Two of these PD neurons ($PD_{in}$) innervate intrinsic muscles p.4, p.7, and p.10 (both attachment points on ossicles of the pyloris) of the pyloric region that are innervated by PY neurons in Panulirus. Two PD neurons ($PD_{ex}$) are homologous to the PD neurons in Panulirus and innervate extrinsic muscles .

The data that support these conclusions are: a) action potentials arising in any Cancer PD neuron can be recorded extracellularly only in either the nerve innervating the $PD_{in}$ or that innervating the $PD_{ex}$ muscles, b) suction electrode stimulation of the lvn in which all four PD neurons send their axons while recording intracellularly from $PD_{in}$ and $PD_{ex}$ muscles results in four different threshold voltages for the production of excitatory junctional potentials (EJPs), two which evoke EJPs only in the $PD_{in}$ muscles and two which evoke EJPs only in the $PD_{ex}$ muscles, and c) hyperpolarization of any one PD neuron only removes EJPs in either the $PD_{in}$ or $PD_{ex}$ muscles.

Since in Panulirus the PY neurons are glutamatergic and the PD neurons are cholinergic, the transmitter used by the two Cancer $PD_{in}$ neurons was determined. Bath application of $10^{-4}$M carbachol produced a large depolarization and conductance increase in $PD_{in}$ muscles. The amplitude of EJPs recorded in $PD_{in}$ muscles evoked by suction electrode stimulation of the $PD_{in}$ axons was reversibly decreased 60-80% by bath application of $10^{-4}$M tubocurare.

We conclude that these anatomically homologous muscles are innervated by different types of neurons (and thus contract at different times in the motor pattern) in Cancer and Panulirus, and that a switch in neurotransmitter receptivity has occurred in these muscles. Research supported by NS-17813 to E.M.

300.9 MOTONEURONAL AND MUSCULAR CONDITIONAL OSCILLATORS COOPERATE TO ORGANIZE CRUSTACEAN RHYTHMIC PYLORIC DILATIONS. P. Meyrand* and M. Moulins. Lab. de Neurobiologie et Physiologie Comparées, place Peyneau, F-33120 Arcachon, France.

In Crustacea, rhythmic movements of the pyloric chamber are driven by an oscillatory network which consists almost entirely of motoneurons. Among these the 2 PD motoneurons, which innervate the pyloric dilator muscles (cpv), are conditional endogenous bursters (Moulins, M. & Cournil, I. J. Neurobiol., 13:447, 1982); Miller, J. & Selverston, A.I. J. Neurophysiol., 48:1378, 1982) i.e. they express their endogenous oscillatory activity only under extrinsic neuromodulatory influences. We show here that the cpv muscles themselves are conditional endogenous oscillators which can be entrained by the rhythmic output of the PD motoneurons.

After denervation, the cpv muscles of shrimps (Palaemon serratus) and rock lobsters (Homarus gammarus) can show two different states : one where they are quiescent and one in which they continue to contract rhythmically. It is always possibe to switch from the quiescent state to the oscillatory state by bath application of $10^{-6}$M dopamine. In the oscillatory state (occuring spontaneously or pharmacologically induced) each contraction is associated with a slow membrane potential oscillation on which fast spikes are superimposed.

This oscillatory behavior seems to be an intrinsic property of the muscle itself since : (1) the frequency of the oscillations is voltage-dependant ; (2) a brief depolarization of a muscle fibre provokes a full, large and long duration oscillation and (3) the ongoing spontaneous oscillatory behavior can be reset by a brief electrical stimulation of the muscle. Furthermore this oscillatory behavior appears to be conditional in that total isolation by bath application of $10^{-5}$M tetrodotoxin abolishes the oscillations which are restored however by addition of dopamine.

When the cpv muscle is in the oscillatory state its rhythmic activity can be entrained either experimentally by phasic stimulation of the appropriate motor nerve or spontaneously by the endogenous burst activity of the PD motoneurons. It has been shown previously that the PD neurons are themselves entrained by higher order oscillatory interneurons (CP) (Moulins, M. & Nagy, F. J. Physiol. Paris, 78:755, 1983). Our results suggest that the final rhythmic dilatory behavior of the pyloric chamber is organized by a cascade of three endogenous oscillators (a premotor oscillator, a motoneuronal oscillator, and a muscular oscillator) at least two of which are conditional oscillators.

300.10 CHOLINERGIC INPUTS REDUCE A STEADY OUTWARD K+ CURRENT ALLOWING ACTIVATION OF A CA2+ CONDUCTANCE WHICH UNDERLIES THE BURST-GENERATING OSCILLATIONS IN LOBSTER PYLORIC NEURONS. F. Nagy, J.A. Benson and M. Moulins Lab. de Neurobiologie et Physiologie Comparées, place Peyneau F-33120 Arcachon, France.

The rhythmic activity of the pyloric neuronal network of the Cape lobster (Jasus lalandii) is due to the ability of the neurons to produce slow, regenerative oscillations which underly their bursting discharge. This ability depends on neuromodulatory inputs from premotor centers. One of these inputs (APM, the Anterior Pyloric Modulator neuron) is cholinergic and involves muscarinic receptors (Nagy, F. and Dickinson, P.S., J.exp.Biol., 105:33, 1983). It was shown previously that the capacity to produce rhythmic slow oscillations results from the activation of voltage-dependent Ca2+ channels (Nagy, F., Benson, J.A. and Moulins, M., Soc. Neurosci. Abs., 10:148, 1984). We now present evidence that this activation is indirect and depends on the reduction by cholinergic inputs of a steady outward current. After deafferentation of the stomatogastric ganglion (which contains the pyloric network) by bath application of $10^{-7}$M tetrodotoxin (TTX) the slow regenerative rhythmic oscillations of the pyloric neurons disappear. Superfusion with oxotremorine, a muscarinic agonist, induces a steady depolarization and subsequently restores the slow regenerative oscillations. In the presence of Ca2+ channel blockers (Mn2+ and Cd2+), oxotremorine does not provoke the regenerative events but still causes the same amount of steady depolarization. Since this depolarization is insensitive to TTX and Ca2+ channel blockers, the possibility of a reduction in a steady outward K+ current by the muscarinic agonist was investigated. In earlier experiments we injected TEA, a K+ channel blocker, into one of the pyloric pacemaker (PD) but this treatment does not mimic the effects of oxotremorine i.e. does not evoke the slow regenerative oscillations. However, the PD neuron is one of a group of three strongly electrically coupled pacemakers : it is thus possible that the membrane of the single TEA-injected neuron was held at a constant potential (subthreshold for oscillation) by the two non-injected neurons. TEA was therefore injected into all three neurons simultaneously. In the presence of TTX the injected neurons then exhibited regular oscillations. Thus blockade of the K+ channels of the neurons (by intracellular injection of TEA) mimics the action of oxotremorine. The oscillations induced by TEA are still blocked by Mn2+. However the three neurons are depolarized and addition of oxotremorine does not depolarize the neurons further. This suggests that induction of oscillations by either TEA injection or oxotremorine exposure occurs via the same mechanism, namely a reduction in a steady K+ conductance. From these observations, we propose that activation of muscarinic receptors of the pyloric neurons reduces a substhreshold outward K+ current allowing activation of a voltage-dependent Ca2+ current which causes the production of burst-generating oscillations.

300.11 GABAERGIC INACTIVATION OF BURST GENERATING OSCILLATIONS IN LOBSTER PYLORIC NEURONS. J.R. Cazalets*, I. Cournil* and M. Moulins (SPON: F. Nagy). Lab. de Neurobiologie et Physiologie Comparées, place Peyneau F-33120 Arcachon, France.

Rhythmic motor output of the rock lobster pyloric network of the stomatogastric ganglion (STG) results from the ability of the neurons to produce slow regenerative oscillations which underly their firing in periodic bursts. However these properties, although endogenous, are expressed only under the influence of neuromodulatory inputs descending from premotor centers. Until now all such modulatory influences described cause activation of the oscillatory behavior of the pyloric neurons (see Nagy, F. et al., Abstract this meeting). Here we show that inactivation of the oscillatory behavior can be produced by GABAergic inputs to the STG.

Bath application of $10^{-4}$M GABA to the STG suppresses burst generating oscillations in all the pyloric neurons, i.e. the pyloric dilator neurons no longer produce oscillations while the pyloric constrictor neurons are no longer able to generate plateau potentials. The same effects can be obtained by bath application of $10^{-5}$M muscimol, a GABA agonist. Conversely prior application of GABA antagonists (bicuculine, picrotoxin) protects the pyloric neurons against GABA exposure. All these pharmacological effects are reversible. Moreover GABA seems to act directly on the pyloric neurons themselves. After isolation of the STG neurons by bath application of $10^{-6}$M tetrodotoxin and subsequent induction of oscillations in the pyloric neurons by bath application of a muscarinic agonist ($5.10^{-6}$M oxotremorin, see Nagy, F. and Dickinson, P.S. J. exp. Biol. 105:33, 1983) GABA superfusion of the STG still causes suppression of oscillation.

Parallel to this pharmacological study we have obtained evidence which shows that the STG receives GABAergic inputs. Firstly, an immunohistochemical study using an antibody against GABA reveals immunoreactive fibres in the single input nerve to the STG and profuse ramification of axon terminals in the STG neuropile. Moreover no cell body in the STG appears to be GABA immunoreactive. Secondly, a radio-enzimatic assay demonstrates a strong glutamate decarboxylase activity in the STG (10 times higher than in the remainder of the nervous system) further suggesting that GABA is synthesised in the STG.

These results suggest that GABAergic inputs to the STG are able to inactivate the burst generating oscillations of the pyloric neurons. Thus the pyloric neurons appear to be exposed to two different neuromodulatory influences : inductive inputs which are able to unmask their oscillatory behavior and suppressive inputs which are able to mask their oscillatory behavior.

300.12 OPERATION OF THE STOMATOGASTRIC NERVOUS SYSTEM IN SITU: MODULATION AND FUNCTION OF PYLORIC MOTOR PATTERNS. B.C. LaMon and J.P. Miller. Zoology Dept., University of California, Berkeley, 94720.

The stomatogastric nervous system of the spiny lobster, Panulirus interuptus, has received intensive investigation as a model system for the study of pattern generation. The stomatogastri ganglion(STG) is located in the lumen of the anterior aorta and contains the cell bodies of two central pattern generators(CPGs) that control the activities of the gastric mill and pylorus regions of the foregut. Studies utilizing isolated ganglion preparations have recently characterized the network connectivity and intrinsic cellular properties of the stomatogastric CPGs. We have now developed an in situ preparation from which we can record the motor patterns of the pyloric CPG with all sensory, CNS and neuromuscular connections intact. In order to maintain a viable preparation it is necessary to cannulate and perfuse the anterior aorta with fresh saline solution. By appropriate selection of multiple recording sites, the activity of the motorneurons innervating each of the muscles involved in pyloric function can be separately identified. Comparison of in situ and isolated ganglion motor patterns shows that the patterns are qualitatively similar with respect to the sequence of neuromuscular activity. However, in situ preparations reveal a far greater repertoire of motor patterns involving substantial variation in cycle frequency, burst parameters(phases,durations, spike frequencies) and the number of cell types participating in the rhythm. We will describe a previously unreported modulation of the pyloric pattern which occurs during midgut peristalsis as well as pattern alterations produced by stimulation of sensory nerves. Systematic changes in perfusion rate, temperature and oxygenation were also found to have substantial pattern effects, indicating that experimental control of these variables is important for physiological studies of the pyloric CPG. Diagrams presenting the anatomy and mechanics of the pylorus will describe the function of pyloric movements. The expression of different motor patterns by the pyloric CPG will be evaluated with respect to the physiological mechanisms proposed to account for pattern generation in this system. (Supported by grants NIH-NRSA NS-06979 and NSF BNS-8202416.)

300.13 VOLTAGE CLAMP STUDIES OF ISOLATED SOMATA FROM IDENTIFIED PATTERN GENERATOR NEURONS IN THE LOBSTER STOMATOGASTRIC GANGLION.
D.K. Hartline, J.Y. Palacio* and D.V. Gassie*, Bekesy Lab, Univ. of Hawaii, Honolulu, HI 96822

We extended the study of Graubard and Hartline (Soc. Neurosci. Abstr. 10:1073, 1984) to ligatured pyloric somata using a 2-microelectrode voltage clamp. Somata had rest potentials more negative than intact neurons, and input resistances up to 25 Mohms (3-6 Mohms more usual). They were devoid of obvious inward current but exhibited at least 3 outward currents: 1) $I_J$: $Ca^{++}$ dependent inactivating current elicited from a standard hold of -50(mV) by test steps above -25; magnitude up to 500 nA at 0 mV; inactivation increased for holds from -45 to 0 (+H, Fig. B); 2) $I_A$: $K^+$ dependent inactivating current activated by steps above -45 from hold levels or 200 msec conditioning pulses below -50; magnitude up to 600 nA at 0 mV; inactivation increased with holds from -90 to -50 (-H, Fig. B) 3) $I_K$: inactivation-resistant current activating slowly above -25 mV and persisting when $I_J$ is eliminated; magnitude up to 100 nA at 0 mV. Fig. A shows activation, B inactivation of these currents.

$I_J$ was very susceptible to elimination or permanent inactivation in damaged somata. Decreases in $I_J$ by blockers or inactivation were frequently associated with increases in $I_A$. Two time-constants of inactivation of $I_A$ (ca 30 and 300 msec) and at least 2 for $I_J$ (ca 30 and 3000 msec) were observed. $I_A$ was particularly prominent in PD and some PL somata; it was small in IC, VD, and some LP somata. The slowly-inactivating component of $I_J$ was prominent in PD; the rapidly-inactivating component in "followers" (LP, PL, PE, IC, VD). The residual $I_K$ was more prominent in LP than in PD. Differences in prominence of $I_A$ may contribute to differences in phase relations in the patterned output. $I_J$ may control depolarization during plateaus.

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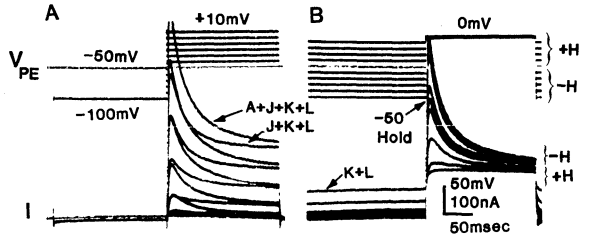


300.14 INTRACELLULAR ANALYSIS OF UROPOD STRETCH RECEPTOR INPUT IN THE TERMINAL GANGLION OF THE CRAB EMERITA ANALOGA.
D. H. Paul. Biology Department, University of Victoria, BC V8W 2Y2

Extracellular recordings from roots of the 6th abdominal ganglion of Emerita revealed complex motor reflexes elicited by tension applied to the uropod stretch receptors, of which 4 non-spiking sensory receptors (NSSR) constitute the principle component. Injection of long current pulses intracellularly into individual NSSRs produced qualitatively similar motor reflexes (Paul, Science 176: 680-682). This observation suggests that input from all 4 NSSRs converges on the same set of premotor interneurons that orchestrate the reflexes. Furthermore, one interpretation of results of other experiments, in which electromyograms associated with uropod beating were compared before and after bilateral receptor ablations, is that those neurons coordinating the reflexes are themselves part of the central pattern generator (Paul, J. exp. Biol.65: 243-258; J. Neurobiol.10: 273-289). In order to test this hypothesis I have begun to trace the afferent signals through the ganglion by recording intracellularly from and injecting dye into neurons whose activity is affected by stretch applied to the uropod receptors. These include motoneurons (MN) and local interneurons (IN). Simultaneous intracellular recordings from an NSSR and certain MNs reveal that polarization of the MNs' membranes is very sensitive to the amount of tension on the receptor strand. Some, including the power-stroke excitor and return-stroke peripheral inhibitor, depolarize, whereas others, including one of the return-stroke excitors, hyperpolarize in parallel with depolarization of the recorded NSSR, there being no apparent threshold for these responses. Whether any of the MN depolarizations are mediated monosynaptically is not yet known. Among the INs, one spiking bilateral neuron has a particularly provocative behavior. It receives tonic IPSPs from an unidentified source that appear to be modulated in frequency by NSSR input; however, the neuron also undergoes apparently smooth hyperpolarizations during stretch of the receptor ipsilateral to its soma. Following release from hyperpolarization, caused either by the afferent input or by current injection, this IN generates trains of bursts (3 to >30 bursts/hyperpolarization, N=7; periods/train of 0.3±0.1s to 2±1s, N=87). This post-hyperpolarization bursting does not elicit motor output, but it could provide phasic reafference to the pattern generator during uropod beating. It might also contribute to generation of the rhythmic motor pattern in isolated nerve cords if the frequency of the IPSPs the neuron receives were modulated during this activity; and if this were the case, the neuron's structure would suit it for a role in bilateral synchronization of motor output.

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300.15 DUAL ACTION OF SEROTONIN ON SWIMMING IN TRITONIA.
P.A. Getting, A.D. McClellan and M. Li*. Department of Physiology & Biophysics, University of Iowa, Iowa City, IA 52242.

To assess the potential involvement of various neurotransmitter substances in the generation and modulation of escape swimming in Tritonia, we tested the effects of 12 pharmacological agents. Substances were injected into the hemocoele of whole animals or were bath applied to isolated brain preparations. Swimming was elicited in the whole animal by application of 1 ml of 3M NaCl to the dorsal epithelium or, in the isolated brain, by electrical stimulation of a peripheral nerve. For the behavioral experiments, swimming was quantified by counting the number of ventral flexions. In isolated brain preparations, intracellular recording from flexion neurons was used to assess swim activity. Four agents (glycine, histamine, proctolin, and FMRFamide) had no effect on swimming at concentrations up to $10^{-3}$M. Swimming was inhibited by 7 other agents (dopamine, octopamine, aspartate, glutamate, carbacol, pilocarpine, and $SCP_B$).

Serotonin (5-HT) had a dual effect on swimming. At concentrations above $10^{-3}$M, 5-HT application elicited a swim within two minutes in the absence of peripheral stimulation. This initial swim bout was followed by a prolonged period of inhibition lasting up to 24 hrs. The extent of the inhibition was dose dependent with a threshold of $5 \times 10^{-6}$M and a maximal effect at $10^{-3}$M. At the higher concentrations, swim activity was completely blocked. The 5-HT antagonist, methysergide (10ug/ml), caused a significant reduction in the number of cycles per swim and in the extent of the dorsal flexions. Injection of 5,7-dihydroxy-tryptamine at doses of 22-220 ug/gm body weight also caused a marked reduction in both the number of cycles and the magnitude of the dorsal flexions. These data suggest that the activation of 5-HT receptors was sufficient and necessary for the expression of the normal swim pattern.

Potential serotonergic neurons were localized using an antibody to 5-HT (Immuno Nuclear Corp)(rhodamine labelled IgG). Five neurons in the region of the swim pattern generator interneurons were stained by this antibody. Intracellular injection of identified neurons with Lucifer yellow prior to antibody staining indicated that 3 of the 5 neurons were dorsal swim interneurons (DSI). These interneurons form a necessary part of the central pattern generator network for swimming (Getting, P.A. et al., J. Neurophysiol., 44, 151-164, 1980) and appear to use 5-HT as a transmitter. Supported by NIH research grant NS17328.

300.16 INHIBITORY MOTOR NEURONS ARE PART OF THE NETWORK WHICH GENERATES LEECH (HIRUDO) SWIMMING ACTIVITY.
W. O. Friesen. Department of Biology, University of Virginia, Charlottesville, VA 22901.

Three essential properties characterize all elements of neuronal network oscillators: 1) membrane potential oscillations are present, 2) intracellular current injection acts to shift the activity phase of the other elements in the circuit and 3) connections occur with other neurons of the oscillator circuit. Leech oscillatory interneurons described previously exhibit all three properties and are therefore considered to be members of the network generating the swimming rhythm (Friesen, Poon and Stent, J. Exp. Biol. 75, 1978; Weeks, J. Comp. Physiol. 148, 1982; Friesen, J. Comp. Physiol. 156, 1985). A motor neuron, cell 1 (a Dorsal Inhibitor 'DI'), which exhibits the first two properties was not considered previously to be a member of this network. Recent experiments show that cell 1 also exhibits the third property; namely, cell 1 (and a second DI, cell 102) displays synaptic interactions with cell 115, a recently discovered oscillatory neuron of the swim network.

Cell 115 is a paired intersegmental interneuron found on the dorsal aspect of most (perhaps all) segmental ganglia. It exhibits all three properties of oscillator elements. 1) Its soma potential undergoes 10 mV oscillations which are phase-locked ($0^o$ phase) to oscillations in the swim network. 2) Injection of depolarizing current into cell 115 shifts the phase of the swimming rhythm. 3) Cell 115 exhibits strong (probably monosynaptic) inhibitory interactions with cell 28 and receives strong (probably monosynaptic) excitatory input from cell 208, both of which are neurons in the swim network. Cell 115 also receives monosynaptic excitatory input from swim-initiating neurons (cells 204/205).

The two DI motor neurons are linked to cell 115 via strong reciprocal inhibition, thus forming a subcircuit which could contribute to oscillation generation. The inhibitory connections of DI neurons to cell 115 also provide a pathway by which depolarization of these cells can act to shift the phase of the swimming rhythm. Because of these new results, the DI motor neurons must be considered elements of the network which generates the swimming rhythm of the leech. Therefore this oscillator network includes both interneurons and inhibitory motor neurons. Supported by NSF grant BNS81-10243.

301.1 SIZES AND INTRANUCLEAR DISTRIBUTION OF RUBROSPINAL NEURONS IN THE RAT. D. Bulyalert* and D.R. Humphrey (SPON: D.J. Reed). Lab. of Neurophysiology, Emory Sch. of Med., Atlanta, GA 30322.

On the basis of relative cell sizes, the red nucleus of the monkey, the cat and the rat can be divided into a rostral parvocellular and a caudal magnocellular division. In the monkey, rubrospinal fibers originate exclusively from medium and large sized neurons in the magnocellular division (Smith & Courville, Neurosci. Abstr., 2: 551), whereas in the cat, rubrospinal fibers originate also from small and medium sized neurons in the parvocellular division (Pompeiano & Brodal, J. Comp. Neurol., 108: 225). In the rat, rubrospinal fibers are known to originate from all portions of the nucleus (Shieh et al, J. Comp. Neurol., 214: 79), but the relative contribution from neurons of different sizes and their intranuclear distribution have not been studied in detail. It is the purpose of this presentation to provide such data.

Injections of 1% WGA-HRP were made into the cervical (C4, N=2) or lumbar (L3, N=2) spinal cords of adult, Long-Evans rats. After survival periods of 48 hr, the animals were perfused transcardially with saline, followed by 2.5 % glutaraldehyde in phosphate buffer. The tissue was processed with a modification of the TMB method developed by Mesulam (Gibson et al, Brain Res., 298:235), and all sections were counterstained with thionin. Cell sizes were measured only in labeled neurons with visible processes and nuclei. Our major findings were as follows. (1) As reported for other species, cells projecting to cervical levels were found dorsomedially within the nucleus, whereas those projecting to lumbar levels were found ventrolaterally. (2) Projections to both the cervical and to the lumbar level originate, however, from the entire rostrocaudal extent of the nucleus, with an increasing density of projection neurons in the rostral-to-caudal direction. (3) Cells of all sizes are found throughout the nucleus, but small (soma dia. $<20\mu$) to medium (20-25$\mu$) cells predominate in the rostral one-third, medium-sized cells in the middle one-third, and large (26-40$\mu$) to giant-sized ($>40\mu$) cells in the caudal one-third of the nucleus. (4) At any rostrocaudal level within the red nucleus, the size distribution of labeled rubrospinal neurons was similar to that for all nuclear cells found at that level; i.e., projections originate from cells of particular sizes in proportion to their numerical density at that rostrocaudal level. (5) At any rostrocaudal level, however, the average or median size of neurons projecting to lumbar levels is slightly greater than that of neurons projecting to cervical levels. (Supported by NIH Grant NS 10183).

301.2 IDENTIFICATION OF SENSORY AFFERENTS TO RED NUCLEUS AND THEIR SPINAL PATHWAY, IN THE CAT. D. Bourbonnais* and Y. Padel. Lab. of General Neurophysiology, CNRS, INP, B.P. 71, 13402 Marseille Cedex 9, France.

In mammals, the caudal red nucleus (RN) receives converging influences from both the contralateral nucleus interpositus of the cerebellum and from the ipsilateral motor cortex. It has been shown that the RN receives a third input from the periphery via fibres or collaterals of fibres running in the dorsal columns of the spinal cord (Padel Y. & Jeneskog T., Neurosci. Lett., 21:177-182, 1981). The present experiments were designed to identify the types of primary afferents responsible for the sensory responses in red nucleus cells and to locate the spinal pathway relaying the dorsal column fibres.

Cats were anaesthetized with chloralose (60 mg/kg I.V.), decorticated and the brachium conjunctivum was cut stereotaxically in order to remove cerebral and cerebellar inputs to red nucleus. The rubrospinal neurones were intracellularly recorded with glass micropipettes filled with saturated solution of $Na^+$ citrate.

When graded stimulation was applied to cutaneous nerves, the responses of RN cells started with low current (1.5 T) and their amplitudes increased with currents reaching 10 T. This indicates that different types of cutaneous receptors contribute to the red nucleus response. When muscular nerves were stimulated, currents over 2.5 T were necessary to obtain responses. This seems to exclude any participation of group I afferents.

Large transversal lesions of the dorsal part of the spinal cord at cervical level did not prevent the sensory responses in red nucleus cells. Moreover, systematic mappings with microstimulation of the spinal cord and brain stem showed that the sensory pathway runs ventromedially in the spinal cord and that its fibres are intermingled with lemniscal fibres in the brain stem. However, repeated spinal stimulation induced a progressive decrease of the amplitude of the responses, although brain stem stimulation gave short latency monosynaptic EPSPs. This indicates that at least some of the spinal afferents are not projecting directly to the red nucleus. They are relaying in the upper spinal cord or in the caudal brain stem at synapses subjected to inhibitory influences.

Study supported by a CNRS grant ASP n° 387 and an MRC fellowship to D. Bourbonnais.

301.3 LOCALIZATION OF SEROTONERGIC MEDULLARY RAPHE NEURONS PROJECTING TO THE LUMBAR SPINAL CORD IN THE CAT. S.J. Fung, V.K. Reddy,* R.M. Bowker and C.D. Barnes. Dept. of VCAPP, College of Veterinary Medicine, Washington State Univ., Pullman, WA 99164

Serotonin-immunoreactive (5-HT) neurons have been localized in the medullary raphe nuclei of the cat (Jacobs et al., 1984) and so also the projections of the medullary raphe neurons to the spinal cord have been demonstrated by retrograde labeling with horseradish peroxidase (Martin et al., 1978). However, 5-HT neurons of the medullary raphe nuclei of the cat that project to the lumbar spinal cord have not been localized. These neurons were observed using a retrograde transport method combined with immunocytochemical techniques.

Laminectomy was performed in anesthetized cats at vertebra L5 and a 25-50% solution of HRP was injected bilaterally into the $L_7$ segment of the spinal cord. Following a 3-day survival period, the animals were reanesthetized and perfused with physiological saline followed by 3.8% paraformaldehyde in 0.1M phosphate buffer. 30% buffered sucrose solution was infused after fixation to prepare the brain stem for sectioning. The brain stem was sectioned on a freezing microtome followed by reacting the sections in 3,3' diaminobenzidine hydrochloride and $H_2O_2$ after preincubating them in 0.5% $CoCl_2$. The sections were then incubated in serotonin antiserum (1:3,000 dilution) and were further processed by immunoperoxidase method.

In the cats, 5-HT neurons projecting to the lumbar spinal cord contained homogenous brown staining cytoplasm and the HRP reaction was seen as black punctate granules distributed throughout the soma and the proximal parts of the dendrites. These double labeled cells (5-HT and HRP) were small to medium sized neurons and were either fusiform or multipolar in shape. They were observed in all three medullary raphe nuclei-nucleus raphe obscurus, nucleus raphe pallidus and nucleus raphe magnus. A few multipolar neurons exhibited HPR reaction product but did not stain for serotonin. In representative sections, the numbers of spinally projecting neurons in the raphe nuclei were counted. These preliminary counts suggest that 69.0% (96 of 139 neurons counted) of the HRP filled neurons also stained for serotonin immunoreactivity. These observations correlate well with similar findings in monkey and rat (Bowker et al., 1982; 1983).

Bowker, R.M. et al., *Brain Res. Bull.*, 9 (1982) 271-278.
Bowker, R.M. et al., *Brain Res.*, 288 (1983) 33-48.
Jacobs, B.L. et al., *Brain Res. Bull.*, 13 (1984) 1-31.
Martin, R.F. et al., *J. Comp. Neur.* 182 (1978) 77-88.
Supported by NS-20979-02 and NS-22321.

301.4 CYTOARCHITECTONIC/CYTOCHEMICAL FEATURES OF THE NUCLEUS TEGMENTI PEDUNCULO-PONTINUS (NTPP) IN THE RAT ARE DISTINCT FROM 'EXTRAPYRAMIDAL' PROJECTIONS TO THE MESOPONTINE TEGMENTUM. D.B. Rye, C.B. Saper, and B.H. Wainer. Dept. Pharm. and Phys. Sciences, Univ. of Chicago, Chicago IL, 60637.

A cytoarchitectonic delineation of the NTPP has been established only in the primate brainstem. Terminal labeling of nigral and other 'extrapyramidal' efferents to the tegmentum has been associated with the NTPP in the feline and rodent. A correlation between the area of the tegmentum in receipt of these projections and any recognizeable cell group(s) (i.e. the NTPP) has not been established. Since previous delineation of the nuclear pattern of the mesopontine tegmentum has engendered considerable inconsistency and confusion, the cytoarchitecture and cytochemistry of the NTPP was investigated employing coronal, horizontal and sagittal sections of the rat brainstem stained for Nissl substance alone, and in combination with choline-acetyltransferase (ChAT) and/or tyrosine-hydroxylase immunohistochemistry. Cytoarchitectonic criteria, the presence of large, multipolar cholinergic neurons, and other cytochemical features distinguish the NTPP from the substantia nigra (SN)/A9 and retrorubral field (RRF)/A8 rostrally, cells embedded within the decussation of the brachium conjuctivum (DBC) medially, lateral lemniscal nuclei laterally, the nucleus cuneiformis dorsally, the pontine oralis ventrally, and the parabrachial nucleus caudally. Nigral and pallidal efferents were then labeled employing autoradiographic tracing or WGA-HRP in combination with ChAT immunocytochemistry, in order to establish their relationship to the NTPP. In both cases, labeled fibers exit the SN laterally and immediately assume a position in the RRF/A8, dorsomedial to NTPP. Fibers continue through the DBC medial to the middle third of the NTPP, and encounter only a medial portion of the NTPP in its caudal third. Labeled fibers were conspicuously absent within a caudal, dorsolateral compact portion of the NTPP. In summary, the NTPP is a readily demarcated nucleus within the rat brainstem comprising a population of cholinergic neurons that is not completely delimited by nigral or pallidal efferents. Supported by The McKnight Foundation and USPHS 5T32-GM07281, HD-0453,NS-17661,NS-00631,and NS-18669.

301.5 ORGANIZATION OF CORTICORETICULAR PROJECTIONS IN THE RAT. D.B. Newman*, Department of Anatomy, Uniformed Services University, Bethesda, Maryland 20814-4799.

A mounting body of evidence suggests that the mammalian brain stem reticular formation exhibits an organization which is far more complex than previously thought. Recent studies by this investigator suggest, for example, that at least twenty-six brain stem reticular nuclei or subnuclei of the rat send fibers to the spinal cord. These nuclei can be distinguished on the basis of several criteria, particularly cytoarchitectonics and dendroarchitectonics. The variegated dendritic ramification patterns observed when comparing one reticular nucleus with another suggests that these reticular nuclei may receive distinct or characteristic sources of afferent inputs. To test this hypothesis, one class of reticular afferent systems, namely, corticoreticular fibers, were examined in detail. Small volumes (900 nl) of 1% horseradish peroxidase-wheat germ agglutinin (HRP-WGA) conjugate were stereotaxically injected into various cortical regions of anesthetized adult albino or hooded rats. The animals were allowed to survive two to three days, and then were anesthetized and perfused with aldehyde fixatives. The tissue was reacted with tetramethylbenzidene histochemistry and examined with polarized light microscopy for the presence of HRP-WGA-filled corticoreticular terminals. The data to date suggest that corticoreticular fiber systems exhibit a high degree of specificity, i.e., certain reticular nuclei or subnuclei receive a much heavier corticoreticular input than others. Furthermore, the various corticoreticular fiber systems tend to respect the reticular nuclear or subnuclear boundaries delineated previously by this author on the basis of cytoarchitectonic or dendroarchitectonic criteria. Finally, the pattern of corticoreticular labeling varies when comparing one cortical injection site, e.g., motor cortex, with another, e.g., somatosensory cortex. Taken together, these data lend credence to the hypothesis that the mammalian brain stem reticular formation is a heterogeneous, highly complex assemblage of discrete nuclei, each with its own particular cytoarchitectonics, hodology and function. (Supported by USUHS Grant R07059.)

301.6 CONTROL OF ATONIA ELICITED BY STIMULATION OF THE MEDIAL MEDULLA. Y. Y. Lai and J. M. Siegel. Neurobiol. Res., VAMC Sepulveda, CA 91343 and Dept. of Psychiatry, UCLA School of Medicine, Los Angeles CA 90024.

Stimulation of the medial medullary reticular formation (MMRF) produces atonia of the antigravity muscles in decerebrate animals. Activation of this area is believed to be responsible for muscle atonia during REM sleep. We have found that MMRF-induced muscle atonia can be inhibited or reversed by administration of sodium nitroprusside, which decreases systemic blood pressure. The present experiment was aimed at further investigating the mechanisms controlling the MMRF-induced muscle atonia.

The experiment was performed on decerebrated, unanesthetized cats of either sex, weighing 2.5-4.0 kg. The animals were decerebrated at the precollicular level under halothane-oxygen anesthesia. Balloon tipped catheters were inserted into the descending aorta and inferior vena cava for nonpharmacological manipulation of blood pressure. Bipolar electrodes were implanted into the splenius, occipitoscapularis, and triceps brachii muscles bilaterally. A stainless steel monopolar microelectrode or concentric bipolar electrode was placed in the medial medulla at P 8.0 to 13.0, L 1.0, and D -6.5 to -9.0. The stimulation frequency and intensity was varied from 20 to 150 Hz and 10 to 800 uA, respectively. A 300 msec train of 0.2 msec rectangular pulses was delivered to MMRF. Electromyograms were recorded by a Grass polygraph and digitized for computer analysis. Pulse triggered averaging was used to analyze the effect of MMRF stimulation on neck muscle activity.

The results showed: 1) there is an optimal stimulation intensity at each frequency for the MMRF-induced muscle atonia. Optimal current levels ranged from 30 to 100 uA. 2) the inhibitory effect of MMRF stimulation on muscle activity is more prominent in the contralateral side than in the ipsilateral side ($p < 0.01$, sign test). 3) Decreasing the blood pressure by inflation of a balloon in the inferior vena cava induced the reversal of response of muscle tone to the MMRF stimulation, while increasing the blood pressure by occlusion of the descending aorta had no effect. Therefore, the reversal response can be induced by blood pressure reduction, and does not require any other pharmacological action of sodium nitroprusside.

These results lead us to hypothesize that two intermixed populations of neurons, having inhibitory or facilitatory effects on muscle tone, are present in the MMRF. These cell groups have different sensitivity to electrical stimulation and have opposite response to blood pressure change.

301.7 HETEROGENEITY OF RETICULAR FORMATION PROJECTIONS FROM THE MEDULLA TO THE DIENCEPHALON. R. Waltzer* and G.F. Martin (SPON: E. Michal). Department of Anatomy, The Ohio State University, Columbus, Ohio 43210.

The study reported here was designed to determine whether connectional heterogeneity is characteristic of the projections from the medullary reticular formation (RF) to the diencephalon of the North American opossum. Three experimental paradigms were used to address this question. In the first, large injections of horseradish peroxidase (HRP) were made into the diencephalon of anesthetized opossums. Such injections labeled neurons in all nuclei of the medullary RF and raphe except for the raphe pallidus. These results provided a framework for the second paradigm in which we examined autoradiographic cases with injections of 3H-leucine in many of these medullary nuclei. Similarities and differences were noted in the location and density of diencephalic labeling. In all cases orthograde labeling was present within the pretectum (PrT), the parafascicular (PF) and central nuclei of the thalamus as well as the lateral hypothalamic area (HyL) and zona incerta. Labeling in the PrT was dense after injections in the nucleus reticularis gigantocellularis (RGc) and the adjacent nucleus reticularis gigantocellularis-pars ventralis (RGcv), but sparse after injections of the nucleus reticularis lateralis (RL). In contrast, injections of the RL produced the heaviest labeling within medial hypothalamic areas. When the injection included the RL or raphe magnus, autoradiographic labeling was also found in the ventrobasal nucleus of the thalamus. Data derived from these studies provided the basis for the third group of experiments in which injections of HRP or lectins conjugated to HRP were confined to specific diencephalic areas labeled in the autoradiographic cases. Injections which included the HyL or PF resulted in retrograde cell labeling in most nuclei of the medullary RF. In contrast, injections of other diencephalic nuclei often produced more restricted RF labeling. For example, only the RL, RGc, and RGcv were labeled after a PrT injection. Our results suggest that some RF nuclei of the medulla project to widespread areas of the diencephalon but that certain of these projections are more dense in some cases than in others. The connectional heterogeneity observed in these studies may subserve the functional diversity of the RF reported by others (Siegel, Brain RS. Rev. 1:69 '79). It is our intent to utilize opossums, because of their unique embryology, to determine whether the heterogeneity referred to above is present initially in development or is carved out of a more uniform, widespread connectional pattern. Supported by BNS-8309245.

301.8 BRAINSTEM PROJECTIONS OF MOTOR AND SENSORY COMPONENTS OF THE VAGUS NERVE IN THE SQUIRREL MONKEY. M. Kalia and G. Kane-Wanger *. Dept. of Pharmacology, Thomas Jefferson Univ. Sch. of Med., Philadelphia, PA 19107.

The motor and sensory projections of the cervical vagus nerve have been traced in the brainstem of five adult squirrel monkeys using the retrograde and transganglionic transport of horseradish peroxidase (HRP) (Type VI Sigma). Tetramethyl benzidine (TMB) was used as the substrate for HRP histochemistry. Retrogradely labeled neurons were found in the dorsal motor nucleus of the vagus (dmnX), the nucleus ambiguus (nA), nucleus retroambigualis (nRA), the nucleus dorso-medialis (ndm), and the spinal nucleus of the accessory nerve (nspA) (levels 1.5 to -1.6 mm with reference to the obex). In addition, retrogradely labeled neurons were found in the nucleus retrofacialis (nRF) in the rostral medulla (level 1.7 mm) and in the nucleus parabrachialis medialis (nPBM) of the pons (level 3 mm). The existence of these populations of vagal preganglionic motoneurons in the pons have not been described previously in any other species. Transganglionically transported HRP to vagal sensory nerve terminals was identified in various subnuclei of the nucleus of the tractus solitarius (nTS) and the area postrema (ap). In addition a significant number of sensory nerve terminals were found in the nPBM, the nucleus parabrachialis lateralis (nPBL) and the Kolliker-Fuse (KF) nucleus. This is the first anatomical demonstration of vagal afferents terminating in the pons. Previous studies in the cat and the rat have not demonstrated these pontine motor and sensory vagal projections. Examination of serial sections in this study indicates that these medullary and pontine vagal projections form a continuous column through the brain stem. In the squirrel monkey, vagal rootlets enter the brain stem at the ponto-medullary junction which considerably more rostral than in the cat and the rat.

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301.9 **BRAINSTEM DISTRIBUTION OF HORSERADISH PEROXIDASE AFTER INJECTION OF THE CANINE NODOSE GANGLION.**
**N.L. STROMINGER, A.P. KNOX*, D.O. CARPENTER AND D.B. BRIGGS*. DEPT. OF ANATOMY, ALBANY MEDICAL COLLEGE, ALBANY, NY 12208 AND NEW YORK STATE DEPARTMENT OF HEALTH, ALBANY, NY 12201.**

**Horseradish peroxidase conjugated to wheat germ agglutinin (120 ul,5%) was injected unilaterally into the nodose ganglion in a series of dogs. After 48-72 hours, animals were anesthetized and perfused transcardially with heparinized phoshate buffered saline followed by 4-8% gluteraldehyde. Brainstems and cervical spinal cords, blocked transversely, were sectioned at 50 um. Material was reacted with tetramethyl benzidine and stained with neutral red.**

**Dense label ipsilateral to the injection filled the afferent vagal fibers entering the brainstem. This was most distinct near the oral end of the vagal input. The solitary fasciculus contained copious label at all levels. The region immediately surrounding the solitary fasciculus was intensely labeled. This included the areas of the lateral, ventral, dorsal and medial solitary nuclei extending into the region immediately oral to the area postrema (AP) which we designate the pre-area postrema (PAP). Label could be followed almost but not right to the ependymal surface. It is possible that finer terminal ramifications were unlabeled. Label approached the junctional zone of the AP and a few fibers could be followed into it. A small number of labeled fibers were present in corresponding locations contralaterally.**

**Labeled perikarya were entirely ipsilateral to the injection, and were not as numerous as might be expected considering the large number of fibers with chromagen. Label was present in the dorsal motor nucleus of the vagus and in the nucleus ambiguus. Labeled cells also occurred in the PAP as shown previously. In contrast to studies of the origins of vagal efferents (Strominger et al., Neurosci. Abst., 10:331, 1984), the solitary nuclei, especially the ventrolateral subnucleus, contained a good number of filled cells. These tended to be the larger perikarya; reaction product varied from a few scattered particles to intense. In addition, a small cluster of labeled cells were located within the interstices of the spinal trigeminal tract along the course of the entering roots.**

301.10 AFFERENT CONNECTIONS OF THE MAGNOCELLULAR PONTINE RETICULAR FORMATION: A HORSERADISH PEROXIDASE STUDY IN THE RAT. S.J. Shammah-Lagnado*, N. Negrão*, B.A. Silva*, J.A. Silva* and J.A. Ricardo* (SPON: L.R.G. Britto). Dept. of Physiology and Biophysics, Institute of Biomedical Sciences of the University of São Paulo, 01000 São Paulo, SP, Brazil.

As part of a series of studies (Shammah-Lagnado, S.J. et al., Neuroscience, 9:391, 1983, and Neuroscience, 1985, in press) of the fiber connections of the reticular formation, a systematic examination of the nuclei reticularis pontis oralis (RPO) and caudalis (RPC) afferents was undertaken in the rat by the aid of the horseradish peroxidase (HRP) tracer technique. The retrograde markers, either free HRP or HRP conjugated with wheat germ agglutinin (WGA-HRP), were microelectrophoretically ejected and the tissue sections processed according to the tetramethylbenzidine method.

A similar distribution of retrogradely labeled neurons was observed in HRP and WGA-HRP experiments. The RPO appears to receive its main input from the zona incerta and fields of Forel, the superior colliculus, the central gray substance, and the mesencephalic and magnocellular pontomedullary reticular formation. Many other structures seem to constitute only modest sources of RPO afferents; these structures include extensive cortical territories, the nucleus basalis, hypothalamic districts, the anterior pretectal nucleus, the substantia nigra, the accessory oculomotor and the deep cerebellar nuclei, trigeminal, parabrachial and vestibular sensory cell groups, the nuclei raphe dorsalis and magnus, the locus coeruleus, the dorsolateral tegmental nucleus, and the spinal cord. While the afferentation of the rostral portion of the RPC appears to conform to the general pattern outlined above, some striking differences emerge when the innervation of the caudal district of the RPC is considered; thus, for instance, both spinal and fastigial inputs distribute much more heavily to this specific district than to the remaining magnocellular pontine reticular formation.

The present results may contribute to the elucidation of the anatomical substrate of the functionally demonstrated involvement of the RPO and RPC in several domains that include the regulation of the electrocorticographic activity of the neo- and archicortices, somatic motor mechanisms, autonomic phenomena, and processing of nociceptive as well as other kinds of sensory information. (Supported by FAPESP grant 04-76/1375 and FINEP grant B-43/83/0757.)

301.11 RHOMBENCEPHALIC CELLS THAT PROJECT TO THE INTRALAMINAR THALAMUS IN THE RAT. G.A. Kevetter and A.R. Parrott*. Dept. of Otolaryng. and Anat., Univ TX Med. Branch, Galveston, TX 77550

Cells in the rhombencephalon that project to the intralaminar thalamus were investigated in order to determine the input to the portions of the thalamus associated with affective and motor responses to sensory input. Horseradish peroxidase (HRP; 30% Miles in 0.05 M Tris 0.5 M KCl) was injected ionophoretically (5 μA positive current; 50% duty cycle for 7 minutes) into the intralaminar thalamus and surrounding areas of the diencephalon of albino rats. Injections were centered on the parafascicular-centre median complex. After a 48 hour survival time, the animals were perfused with mixed aldehydes and 50 μm frozen sections were cut. Alternate sections were reacted with tetramethylbenzidine. Cells labeled with HRP were identified using both brightfield and darkfield light microscopy. After injections centered on the parafascicular-centre median complex, labeled cells were concentrated in three major areas in the caudal rhombencephalic reticular formation. These areas were the N. paragigantocellularis lateralis (PGCL), the N. gigantocellularis (NGc) and adjacent N. raphe magnus, and the dorsal N. reticularis pontis caudalis (NPc) especially adjacent to the genu of the facial nerve. Although labeled cells were located bilaterally, more were concentrated on the side contralateral to the injection in PGCL and NPc. Labeled cells were also located in the vestibular nuclear complex and the adjacent N. prepositus hypoglossi and N. supragenicularis. Sensory nuclei, such as the dorsal column and trigeminal nuclei contained few labeled cells after injections of the intralaminar complex. By contrast, after control injection into ventrobasal complex, these areas were heavily labeled. Labeled cells were also observed in the deep cerebellar nuclei and the central N. of the medulla. We calculated the cell areas of cells labeled in the three regions of the reticular formation. NGc contained the largest cells ($\bar{X} \pm SD$ = 346.36 ± 172.32, n = 31), of the three areas. However, these projection neurons are not the largest cells in the nucleus. The cells labeled in PGCL ($\bar{X} \pm SD$ = 226.12 ± 67.16, n = 38) and NPc ($\bar{X} \pm SD$ = 211.25 ± 87.14, n = 50) had similar areas. As NGc receives a spinal input, these studies indicate that the NGc may be one component in a spinoreticulothalamic system involved in affective and motor responses to sensory input, such as nociceptive stimuli. The NPc may provide important input to the intralaminar nuclei for its involvement in eye movement control. (Supported by BRSG SO7-RR5427.)

301.12 **LESIONING OF THE INFERIOR OLIVARY NUCLEUS FACILITATES STRYCHNINE SEIZURE IN THE RAT,** M. Anderson*, E. Chung and M.H. Van Woert (Spon: G.M. Lehrer), Graduate Program in Neurobiology, Departments of Neurology and Pharmacology, Mount Sinai School of Medicine, New York, N.Y. 10029

Systemic administration of the nicotinamide analog 3-acetyl-pyridine (3AP) has been shown histologically to result in complete degeneration of the inferior olivary nucleus (ION)[1], specifically all climbing fiber afferents to the cerebellum,[2] in addition to partial ablation of the nucleus ambiguus. Acute toxicity marked by partial aphagia and stridulous breathing is observed 1-2 days post 3AP injection. Pending survival of the initial insult (survival rates ranged from 34-66%) rats display a generalized ataxia. Earlier work done in this laboratory has implicated intact olivo-cerebellar fibers in a protective capacity for pp-DDT induced myoclonus.[3] Here we examined the effect of 3AP treatment and electrolytic lesions of the ION on tonic-clonic seizures produced by the glycinergic antagonist strychnine.

Male Sprague-Dawley rats weighing 125-150 gm were given a single i.p. dose of 80 mg/kg 3AP 18 days prior to strychnine challenge. Bilateral electrolytic lesions of the ION were made using Konig-Klippel coordinates:AP=+4.0 mm, L=±0.7 mm, V= -10.2 mm and by applying a 120 mV for 10 seconds. Lesions were confirmed histologically. Animals were scored for symptoms of ataxia and abnormal movement (reeling, tremor, spasticity), weight loss and signs of general autonomic impairment using an ataxia rating scale devised for this purpose. Seizures were induced by administration of 1.5 mg/kg strychnine i.p.

| | Average No of seizures | Incidence of seizures | Average Seizure Coefficient[a] | Average Onset Latency[b] |
|---|---|---|---|---|
| Control | .06±.07*** | 1/16 | .2[c]** | 7:25[c] |
| 3AP Lesion | 10.62±2.35 | 12/16 | 3.9±1.0 | 5:15±:40 |
| Control | .46±.24*** | 4/13 | 1.8±0.85* | 5:42±1:46 |
| ION Lesion | 2.15±.68 | 11/13 | 9.5±3.6 | 7:55±1:14 |

a:seizure coefficient = total time in seizure state x seizure intensity, units are I-min.
b:Latency values reflect only animals exhibiting seizure.
c:Only one animal exhibited seizure
*P < .05,**P < .001, *** P < .0005.

Animals were not noise sensitive. There was no difference in $^3H$ strychnine binding in the medulla or $^3H$-GABA binding in the deep cerebellar nuclei in 3AP treated rats. These data indicate that destruction of the olivo-cerebellar pathway lowers threshhold to seizures induced by strychnine.

(Supported by USPHS grant NS 12341)
1: Brain Research 77 (1974) 349-364; 2:Brain Research 77 (1974) 365-384, 3:Neuroscience Letters 24 (1981) 103-108.

301.13 SPONTANEOUS INTRINSIC OSCILLATORY BEHAVIOR IN INFERIOR OLIVE CELLS FROM GUINEA PIG: ITS ORIGIN AND MODULATION. L.S. Benardo and R.E. Foster. Neurological Inst, Columbia-Presbyterian Medical Center, New York, NY 10032 and Neurotoxicology Branch, US Army Med Rsch Inst of Cml Defense, Aberdeen Proving Ground, MD 21010-5425.

The source of oscillatory firing behavior of mammalian inferior olive (I.O.) neurons has been the subject of study for many years. Most recently Llinas and Yarom (J. Physiol. 315:549-584, 1981) studied these cells in vitro and identified two kinds of calcium conductance: 1) a high threshold type present in dendrites and 2) a low threshold type of somatic origin. They proposed that interactions between these two conductances were the basis for the olivary rhythmic potentials seen in vivo, though this type of prolonged, self-sustaining activity was not observed in their studies. In the experiments presented here, we now report on recordings of I.O. cells in vitro demonstrating spontaneous oscillations of the membrane potential. Parasagittal or coronal brainstem slices (350-400 um) were cut by Vibratome (brainstems submerged in oxygenated artificial CSF at 4-6°C during cutting) and immediately transferred to the brain slice recording chamber (Schwartzkroin, Br Res. 128:53-68, 1975). We have recorded from over 100 oscillating cells and they are routinely present in all anatomical subdivisions of the I.O. The frequency of oscillation was 4-13 Hz and the amplitude ranged up to 20 mV, peak-to-peak, at rest potential. In some cells the oscillation was of constant amplitude while in others there was a superimposed rhythmicity consisting of cyclic variability in oscillation amplitude having a period of about 0.5 - 1 Hz. The oscillations responded to intracellularly applied current such that there was attenuation with depolarization and amplification with hyperpolarization. Lucifer Yellow CH injection into such cells revealed their morphology to be typical of I.O. cells and multiple cell labeling from a single injection (i.e. dye coupling) was observed in 50% of cases. The oscillatory behavior could be damped for up to 4 sec or longer by stimulation of the neuropil dorsal to the I.O. Oscillations were blocked when slices were exposed to calcium-free medium containing 2mM $Mn^{2+}$. Oscillations could be synchronous within local aggregates of neurons, as revealed by simultaneous intracellular recordings, but such behavior became out of phase with increasing distance between pairs of neurons. The evidence suggests an intrinsic somatic origin for the oscillatory behavior. Our findings are consistent with, and more fully explain, the I.O. physiology seen in vivo. This study offers support for an intrinsically generated oscillatory mechanism which may be operative endogenously, can be locally synchronous within a neuronal aggregate and may be modulated extrinsically by afferent and/or efferent sources.

301.14 IMMUNOHISTOCHEMICAL LOCALIZATION OF ADENOSINE DEAMINASE IN PREGANGLIONIC PARASYMPATHETIC NEURONS IN THE RAT. E. Senba[1], P.E. Daddona[2*] and J.I. Nagy[1]. (SPON: K.W. CHENG) Dept. of Physiology, Univ. of Manitoba, Winnipeg, Manitoba, R3E OW3, Canada and Dept.of Internal Medicine, Univ. of Michigan Med. Sch., Ann Arbor, MI., 48109.

The enzyme adenosine deaminase (ADA) converts the biologically active compound adenosine to its inactive metabolite inosine. We have previously found that ADA could be detected immunohistochemically in only a limited number of discrete neural systems in rat brain and spinal sensory ganglia (Nagy et al., Science 224 166-168, 1984; Neurosci. lett. 48 133-138, 1984). It was suggested that a relationship may exist between high neuronal levels of ADA and the putative neuromodulatory role of adenosine in the CNS. However, some of the best evidence available for such a role of adenosine is with respect to peripheral autonomic systems (Akasu et al., Nature 311 62-65, 1984; Portter et al., Fed. Proc. 42 1623-1632, 1983). We therefore examined whether brain stem and spinal parasympathetic systems or superior cervical ganglia (SCG) exhibit ADA-immunoreactivity.

In the brain stem an ADA-containing cell group was detected dorsolateral to the rostral part of the facial motor nucleus. ADA-immunostained fibers emerging from these neurons could be followed dorsally and laterally to enter main fiber bundles which emerged from the brainstem just caudal to the facial nerve. It has previously been shown (Contreras et al., J. Comp. Neurol., 190 373-394, 1980 that neurons at this location project to sphenopalatine ganglia which supply parasympathetic fibers to various tissues including the lacrimal glands and nasal mucosa. We found that the ADA-immunoreactive brainstem neurons when stained by the immunofluorescence method were simultaneously labelled with fast blue after injection of this retrograde fluorescent tracer into the sphenopalatine ganglion, thus confirming their parasympathetic nature. No ADA-immunostaining was seen in parasympathetic neurons of the inferior or superior salivatory nuclei. In the spinal cord ADA-immunoreactive cells were observed in the intermediolateral region of the 6th lumbar to 4th sacral segments corresponding to the location of preganglionic parasympathetic neurons. To date, we have been unable to detect ADA-immunostaining in neurons of SCG.

The presence of ADA in at least certain populations of preganglionic parasympathetic neurons is consistent with a possible neuroregulatory action of adenosine in these neurons and with the notion that ADA may reflect such an action.

301.15 THE INTERACTION BETWEEN GABA/BZP AND THE OPIOID SYSTEM IN SPINAL CORD. T. Shibuya*[1,3], Y. Watanabe*[1], T. Matsumiya*[2] and B. Salafsky*[3] (SPON: T. Marczynski). 1. Dept. of Pharmacol., Tokyo Med. Col., Tokyo 160, Japan, 2. Dept. of Pharmacol., Tokai Univ., Sch. of Med., Isehara 259-11, Japan, 3. Dept. of Biomed. Sci., Univ. of Ill. Col. of Med. at Rockford, Rockford, Ill. 61107-1897.

The interaction between GABA/BZP's and the opioid system in the central nervous system may relate to certain physiologic functions (e.g. feeding, convulsion, stress, etc.). Our previous paper (Europ. J. Pharmacol., 96:141, 1983) supported the existence of such a mechanism in the CNS. However, it is not known whether these relationships exist in the spinal cord. More recently, we had reported (Europ. J. Pharmacol., 109:307, 1985) that the proportion and development of BZP receptor subtypes in the spinal cord are quite different from those of other brain regions. In the present study we examined the interaction between the GABA/BZP and the opioid system on c-fiber reflex, the heat evoked discharge and the pinprick discharge. In addition we further investigated the characteristics of "peripheral" BZP receptors in the spinal cord. Electrophysiologic experiments were conducted in spinal and intact cats utilizing previous methodology (Life Sci., 29:1507, 1981) while the details of biochemical studies have been published elsewhere (Japan J. Pharmacol., 36:15, 1984). The administration of diazepam to spinal cats suppressed the c-fiber reflex in a dose-dependent fashion while similar treatment in the intact animal showed attenuation of these reflexes. In the spinal cat, naloxone antagonized the suppressed effects of diazepam on the c-fiber reflex. Additionally, the inhibitory effects of diazepam on the heat evoked discharge of the spinal cat were blocked by small doses of naloxone. Biochemically we have noted that the number of "central" BZP receptors was approximately three times less than that of "peripheral" BZP receptors in rat spinal cord. The effects of Ro-5-4864, a "peripheral" BZP antagonist on neuronal activities in the spinal cord was also studied. Preliminary indications suggest that "peripheral" BZP receptors are involved in interrelationships between GABA/BZP and opioid systems in the spinal cord.

301.16 INVOLVEMENT OF NON-GLYCINE INHIBITION IN SPONTANEOUS, COORDINATED RHYTHMS IN CULTURED NEURONAL NETWORKS Michael H. Droge & Guenter W. Gross, Texas Woman's University, Denton, TX.

Monolayer cultures of dissociated mouse spinal tissue develop spontaneous, persistent rhythmic bursting activity that is coordinated among multiple recording sites (Droge & Gross, 1984,1985). Strychnine ($10^{-6}$ M) typically increases firing frequencies to the point of disrupting pre-test bursting indicating that glycine transmission does participate in the observed rhythms. However, we have also observed that $10^{-6}$ M strychnine can suddenly block activity at one recording site while increasing activity at other simultaneous recordings sites.

Since low dose strychnine specifically blocks glycine synapses, the enhancement of activity is considered a disinhibition of excitatory synapses. Similar results in other systems have been used to support the hypothesis that networks capable of generating rhythm involve recurrent inhibition. The differential effect of strychnine presented here indicates that glycine transmission is not the only inhibitory transmitter involved in pattern generation. Rather, the loss of activity following strychnine administration may represent a disinhibition via glycine that increases activity in other types of inhibitory connections. The nature of this connectivity has yet to be determined since much more detailed information is needed at the level of the individual cells to identify the pattern generating network.

Droge, M.H. & Gross, G.W. (1984) Characterization of multiunit rhythmic bursting developing spontaneously in monolayer networks cultured on multimicroelectrode plates. Neurosci. Abst. 10(1); 13.11.

Droge, M.H. & Gross, G.W. (1985) Development of coordinated multisite rhythms in cultured neuronal networks. Nature, submitted.

302.1 DORSAL ROOT REFLEX DURING LOCOMOTION IN NORMAL AND DECEREBRATE CATS. S.H. Duenas*, G.E. Loeb and W.B. Marks. Lab. of Neural Control, NINCDS, NIH. Bethesda, MD 20205

During fictive locomotion, homonymous monosynaptic reflexes (MSR) produced by triceps surae group Ia afferent fibers are reduced in amplitude during the flexion (F) phase of the step cycle, at which time these group Ia afferents are more excitable by stimulation in spinal cord (Eidelberg et al., adjacent poster). MSR transmission could be modulated by the locomotor pattern generator at presynaptic or postsynaptic levels. The present study was designed to determine whether a similar modulation occurs during normal and decerebrate locomotion and to further assess the importance of presynaptic mechanisms. Presynaptic inhibition can be caused by the depolarizing effects of synapses ending on the terminal arborizations of primary afferents (so-called PAD). In addition to reducing the threshold to microstimulation, such PAD, if large enough, can itself give rise to antidromic spikes known as the dorsal root reflex (DRR). We used chronically implanted nerve cuff and EMG electrodes to stimulate and record MSR and DRR responses in normal walking cats that were subsequently decerebrated at precollicular level (sparing the thalamus) and allowed to walk spontaneously on a moving treadmill belt.

In both preparations the amplitude of the MSR evoked by stimulation of lateral gastrocnemius (LG) nerve and recorded in medial gastrocnemius (MG) nerve (or vice-versa) increased during extensor (E) phase of the step cycle and decreased during flexor (F) phase. In decerebrate cats the MSR in MG nerve was also evoked by stimulation in sciatic nerve. A similar waxing and waning in the amplitude of MSR occurred.

In both normal and decerebrate cats, a DRR was recorded in MG nerve (but not in MG or LG muscle) following the stimulation of LG nerve as well as posterior biceps-semitendinosus nerve. This reflex had a latency of 3-4 msec and occurred in correlation with F phase of the step cycle. In contrast, the MSR was recorded in the homonymous and heteronymous muscles. Its latency was 4-5 msec and its amplitude peaked during E phase. In decerebrate cats DRR could be produced by stimulating LG, sciatic and posterior tibial nerves. These findings suggest that the locomotor program includes substantial phasic depolarization in ankle extensor group I fibers during F-phase of the step cycle. Thus presynaptic inhibition appears to play a significant role in gating transmission in the ankle extensor Ia-motoneuron pathway.

302.2 EXCITABILITY CHANGES IN ANKLE EXTENSOR GROUP Ia AND Ib AFFERENTS DURING SPONTANEOUS FICTIVE LOCOMOTION E. Eidelberg, S.H. Duenas* and P. Rudomin. (SPON: M. Bak) Division of Neurosurgery VAH, San Antonio, Texas 78284; Dept. of Physiology, Biophysics and Neurosciences, CINVESTAV del IPN 07000 Mexico, D.F.

Spontaneous fictive locomotion in immobilized cats is a good model to investigate the action of the central pattern generator on afferent input since there are no phasic changes in the patterns of peripheral sensory inflow. One possible mechanism for regulating the efficacy of group I input is the modulation of afferent fiber membrane potential. Experiments were designed to study the excitability of gastrocnemius-soleus (GS) group Ia and Ib fibers by measuring changes in threshold to microstimulation of the intermediate nucleus in high decerebrate, paralyzed cats. In addition, we studied the amplitude of the GS monosynaptic reflex (MSR) and the excitability of group Ia afferent fibers in the motor nucleus.

The excitability of both types of fibers increased during fictive locomotion and decreased following episodes of locomotion. Excitability changes were cycle- and gait-dependent. An increase in excitability usually occurred during the flexion (F) phase of the step cycle for both types of fibers. During spontaneous fictive locomotion, extracellular potentials (ECPs) were recorded in the intermediate nucleus. Spontaneous ECPs were phasically modulated in correlation to the F-phase of the step cycle. This modulation was superimposed on a more tonic modulation with a time course of tens of seconds.

The amplitude of the MSR evoked by stimulation in lateral gastrocnemius and recorded in medial gastrocnemius increased during the extension phase of the step cycle and after periods of locomotion; MSR amplitude decreased during the F-phase of step cycle. The excitability of Ia afferent fibers in the GS motor nucleus, measured by Wall's method on whole nerve, was similar in both phases of the step cycle. However, there was an increase in excitability during the F-phase in individual Ia afferents.

The present experiments support the idea of a tonic primary afferent depolarization (PAD) of extensor group Ia and Ib fibers during spontaneous fictive locomotion. In addition, phasic PAD seems to occur in relation to different fictive gaits. The ECPs suggest the possibility that this PAD may be potassium-mediated.

302.3 LONG TERM DISCHARGE PATTERN OF MOTOR UNITS. B. Bigland-Ritchie, E. Cafarelli and R. Johansson . John B. Pierce Foundation and Quinnipiac College, New Haven, Ct. 06519.

Action potentials were monitored for periods of 5 to 30 min from single muscle fibers of the extensor digitorum communis, rectus femoris and vastus lateralis muscles using tungsten micro-electrodes. In some records the potentials from up to four different low threshold units could be distinguished by differences in their amplitudes and waveforms. These were then followed simultaneously.

During constant low force contractions firing rates declined with time. They were usually higher when first recruited during ramp force contractions than during subsequent force maintainance. After variable intervals (2-5 min) many units ceased firing but they could be re - recruited if the force was increased slightly. Plots were constructed for each unit of changes in threshold as a function of time. The drop-outs were not due to electrode movement because, in most cases, other units recorded simultaneously continued firing without changes of amplitude or waveform. Some units recommenced firing after drop out even though the force remained unchanged and others fired for only short periods(1-5 min) at various times during the contraction, while still others fired continuously.

After 20-30 min of low force activity the maximum voluntary contraction (MVC) had declined by 30-50%. The mean firing rates between drop outs remained relatively unchanged at about 10-12Hz despite several increments in force. During this time the relative force increased from 10 to 50% of the current MVC.

These results support the early concept of motor unit rotation during long term activity; a concept largely discarded in recent times (Stuart and Enoka, 1984, Clinical Neurosciences (5): 471-518). During fatigue muscle contractile failure in previously active units may be minimized by periods of inactivity. But, as contractile failure develops, this may be compensated by recruitment of new units rather than by rate coding in those units already active. The drop out of motor unit activity may be associated with decreased motor neuron excitability rather than changes in synaptic input. This is suggested by the return of unit activity following a period in which the input drive presumably remained unchanged.

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302.4 INTERACTION BETWEEN STRETCH REFLEX AND 8-12 HZ COMPONENT OF PHYSIOLOGIC TREMOR. R.J. Elble, J.Adelman*, and C. Higgins*. Dept. of Medicine, Southern Illinois U. Sch. Med., Springfield, IL 62708.

Physiologic tremor has two distinct rhythmic components. The most prominent component results from underdamped properties of limb mechanics and controlling stretch reflex and has a frequency that is largely determined by limb inertia, mechanical-reflex stiffness, and reflex loop time. A second component of physiologic tremor has a frequency of 8-12 Hz regardless of limb mechanics and loading, and the origin of this tremor remains a source of continuing controversy. We have measured wrist tremor and forearm EMG in 30 adult controls, ages 18-74, during the maintenance of steady posture against gravity. Only 30% exhibited a prominent 8-12 Hz component whereas all controls exhibited a mechanical-reflex tremor. Five control subjects with and without prominent 8-12 Hz components were then studied using a computer-controlled torque motor and manipulandum. Fifty millisecond torque pulses were applied randomly to the wrist to determine 1) the amplitude and latency of the stretch reflex response and 2) the ability to reset the mechanical-reflex and 8-12 Hz tremors. Spring and inertial loads were used to separate the mechanical-reflex and 8-12 Hz oscillations which were otherwise fused into a single hybrid oscillation. The stretch reflex responses did not differ between the two groups of controls. As expected, the mechanical-reflex tremor was readily reset and augmented by the torque pulses. The 8-12 Hz tremor, by contrast, was disrupted rather than reset by the perturbations. Furthermore, the 8-12 Hz tremor behaved in a manner identical to that of mild essential tremor in 7 patients. We conclude that 1) the 8-12 Hz component of physiologic tremor exhibits marked intersubject variability that is not obviously related to stretch reflex gain; 2) this 8-12 Hz tremor may be a forme fruste of essential tremor, and 3) the 8-12 Hz tremor is distinct from mechanical-reflex oscillations produced by external perturbations.

This study was supported by grants from the NINCDS (RO1 NS20973) and from the American Parkinson Disease Association.

302.5 ACTIVITY OF INDIVIDUAL CAT LATERAL GASTROCNEMIUS AND SOLEUS MOTOR UNITS DURING PAW SHAKES. A.W. English, Emory Univ., Atlanta, GA 30322

Paw shaking is a rhythmic movement which involves rapid alternate flexions and extensions of the foot and is produced by selective recruitment of fast-twitch units in lateral gastrocnemius (LG), without activity of slow twitch motor units in soleus (SOL) (Smith et al, J. Neurophysiol. 43:612-620, 1980). Since some of the compartments of LG contain significant proportions of slow twitch motor units, this study sought to determine whether they are also inactivated during shaking. The activity of single motor units in LG and SOL was recorded using miniature "hatpin" microelectrodes which were implanted into the lateral gastrocnemius-soleus nerve (English, Soc. Neurosci. Abstr. 9:360, 1983). Recordings were made as the cats walked on a treadmill with a piece of cellophane taped to the plantar surface of the foot. Shaking was elicited at irregular intervals during the swing phase of the step cycle. All LG units were activated according to one of two walking task groups: early in the EMG burst, or in a sustained pattern during both muscle lengthening and shortening. None of the SOL units studied displayed the sustained pattern during walking, but units in the early task group were noted. During shakes, all LG units fired very briskly. Discharges were very highly correlated to changes in LG length (Fig. 1). Most SOL units were inactive during shakes, but for those which were active, their firing rates were not impressive, and synchronization to LG length changes was poor. The activity of these SOL units is probably related more to the maintenance of limb position during shaking than to the movements. Since, based on axonal conduction velocity and compartmental location, some of the LG units sampled were slow twitch, they are clearly activated differently than SOL units during shakes but not during walking. Thus motor units of similar physiological type in different synergist muscles are not necessarily strict synergists. Different motor units may belong to similar locomotor task groups but they may be used quite differently during different behaviors.

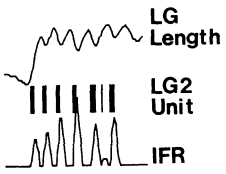

FIGURE 1

302.6 **COMPARISON OF FIRING RATE PATTERNS OF DIFFERENT TYPES OF MOTOR UNITS AT THE SAME RELATIVE TENSION OUTPUT.** B.R.Botterman and T.C. Cope. Dept. of Cell Biology and Anatomy, Univ. Texas Hlth. Sci. Ctr., Dallas, TX 75235.

**Evidence from a variety of experimental approaches suggests that the recruitment and usage of motor units is generally ordered according to their tension output and fatigue resistance. Most studies concerned with the relative fatigue resistance of motor units have been based on one stimulation rate, in which the separation of unit types was stressed. One approach to the study of motor unit fatigue (and potentiation) is to examine the response of different types of units when their tension output is maintained at various levels of maximum tetanic tension (e.g., 15%). In this way, the firing behavior of different units can be compared at the same relative tension output level. Under constant-tension conditions, the firing rate of the motor axon must be adjusted to accommodate the ongoing changes in the tension output of its muscle unit due to the opposing processes of fatigue and potentiation.**

**In 5 cats, motor axons to either the FCR (n=15) or MG (n=24) muscles were isolated from ventral root filaments. Units were classified as type F or S based on the presence or absence of a "sag" profile. A LSI-11/23 computer system was used to maintain a constant-tension output from a unit by changes in its activation rate. This was accomplished via a closed-loop feedback system, in which small deviations in "target" tension resulted in a proportional adjustment of the activation rate of the unit's motor axon.**

**From this approach, it is clear that at low percentages of maximum tetanic tension (<25%) activation rates of type F units dramatically decrease during the contraction, sometimes as much as 50%. Therefore, for many type F units it appears that post-activation potentiation delays the onset of "motor" fatigue. At 15% of maximum tension, two groups of type F units could be tentatively identified. One group could maintain the target tension between 700-2,000 activations, while the other group could do so for >3,500. In addition, it is clear that for many units, particularly type S, constant tension could be maintained for tens of thousands of activations, encompassing a wide range of target tensions (15-75%). It is anticipated that this approach will be helpful in interpreting various recruitment and usage schemes for the three motor-unit types found in most mammalian muscles. (Supported by NIH grant NS17683 to B.R.B.)**

302.7 ELECTROMYOGRAPHIC ACTIVITY OF SELECTED CAT HINDLIMB MUSCLES DURING UNRESTRAINED LOCOMOTION AT VARYING SPEEDS AND GRADES. M.Kuehl*, R.R.Roy, R.J. Gregor and V.R.Edgerton. Brain Research Institute and Kinesiology Dept, UCLA, L.A., CA. 90024.

The duration of electromyographic (EMG) activity of flexors and extensors during unrestrained locomotion is asymmetrical, particularly at slow speeds. To study the asymmetry in more detail, EMG was recorded using chronically implanted intramuscular electrodes from the soleus (SOL), medial gastrocnemius (MG), gluteus medius (GM) and tibialis anterior (TA) muscles of seven adult cats during treadmill locomotion at speeds ranging from .23 to 3.74 m/sec and at grades of 0, 10 and 20°. From the rectified EMG the average cycle period (CP), burst duration (BD) and integral per burst (IA) were determined over a series of 10-20 consecutive steps. Subsequently, mean EMG was calculated as IA/BD, IA per min as step rate*IA per step and 'on-time' per min as step rate per min*BD. The EMG activity to varying speeds and grades was similar in all cats. CP decreased in a hyperbolic fashion with increasing speed for all muscles at any given grade. The BD of the SOL and MG decreased exponentially whereas the BD in the TA remained essentially unchanged or was slightly reduced as a function of speed. The BD of the GM followed the pattern of the SOL and MG at slow speeds, but was longer at speeds greater than 1.17 m/sec. The trends associated with increasing speeds were similar at all grades. Across speeds the IA was relatively constant in the MG, GM and TA, whereas it decreased progressively in the SOL. The IA was elevated at higher grades but at each grade the relationship to velocity was similar. SOL mean EMG did not change at slow speeds but decreased slightly at speeds faster than 2.11m/sec. This finding was most obvious at 10 and 20° incline. In contrast, the mean EMG was elevated in the MG, GM and TA, with the MG showing the largest increase. IA per min was always highest for the SOL followed by the MG and was always the least for the TA. The SOL IA per min decreased at speeds faster than 2.11m/sec. Ankle extensor 'on-time' per min was reduced with increasing speeds as a result of the progressive reduction in BD. In contrast, the TA 'on-time' increased by 150% at the higher speeds. The GM showed a decrease in 'on-time' at the lower speeds, but an increase at speeds faster than 1.17 m/sec. This reflects the slightly longer BD of the GM in comparison to the SOL and MG at the higher speeds. These data are consistent with the hypothesis that as speed increases the level of activity of the motor pools (number of motor units active and/or frequency of excitation) of the MG and GM are increased markedly while the activation levels of the TA and SOL change only slightly.
(Supported by NIH Grant NS 16333)

302.8 Force-Velocity Potentiation in Cat Soleus Muscle During Treadmill Locomotion R.J.Gregor, W.C. Whiting, R.R. Roy, J. Hodgson, R.G. Lovely and V.R. Edgerton. Brain Research Institute and Kinesiology Department, UCLA, Los Angeles, CA 90024

Isometric and isotonic contractions monitored in situ provide selected information on the mechanical properties of a skeletal muscle. Contractions under such controlled conditions however, rarely occur in natural movements and may not directly apply to our understanding of muscle mechanics during locomotion. We have therefore, made direct measurements of soleus muscle force, length and EMG during locomotion and from these measurements examined the force-velocity relationship of the muscle during treadmill locomotion.

Sixty-five step cycles in four cats were analyzed at speeds ranging from 0.8-2.2m/s. Force transducers were surgically implanted on the soleus tendon and bipolar EMG electrodes implanted into the muscle belly. Direct linear transformation techniques were employed using high speed cinematography to estimate muscle length changes in 3D (J. Biomech. 17:685-94, 1984). Data were subsequently smoothed using digital filtering techniques and velocities calculated using finite differences. Terminal experiments provided direct comparison of in situ isometric and isotonic properties with in vivo observations made during locomotion.

Peak force remained relatively constant and time to peak force occurred consistently at 13% of the step cycle at all treadmill speeds. While the magnitude of the stretch fell slightly as speed increased, peak lengthening velocities increased from -46mm/s at 0.8m/s to -77mm/s at 2.2m/s. Peak shortening velocities also increased from 52mm/s at 0.8m/s to 144mm/s at 2.2m/s. Isotonic $V_{max}$ averaged 190mm/s in these animals. At the speeds employed in this study the in vivo force-velocity curve displayed consistently higher forces than those recorded at similar shortening velocities in situ. These higher forces may be due to the stretch imposed on the active muscle in early stance ($E_2$). Preliminary studies on rat soleus, in situ, showed similarly higher forces when the active muscle was lengthened and allowed to shorten. The data suggest that skeletal muscle may be capable of developing considerably more power than that predicted from isotonic force-velocity properties recorded during shortening contractions.
(Supported by NIH Grant 16333)

302.9 KINEMATICS AND DYNAMICS OF THE CAT HINDLIMB DURING LOCOMOTION. G.E. Loeb, W.B. Marks, A.J. Rindos, Jiping He*, W.M. Roberts*, and W.S. Levine*. Lab. of Neural Control, NINCDS, NIH, Bethesda, MD 20205 and Dept. of Electrical Engineering, University of Maryland, College Park, MD 20747

We are developing an analytical model of the cat hindlimb musculoskeletal system. The model presently consists of five two-dimensional segments: toes, foot, shank, thigh, and pelvis. The gross anatomy of 33 muscles with significant action in the sagittal plane has been modeled, including ranges of origin and insertion and radii of pulleys and tethers constraining their tendons. Skeletal motion was derived from digitally smoothed (10 msec time constant) stick figure reconstructions of video stills recorded at 60 fields/s by a high resolution camera with stroboscopic illumination. Ground reaction forces were recorded by a multiaxis force plate: vertical and longitudinal forces and center-of-force of the foot. In the results described below, the skeletal motion was obtained from fast, steady walking on the treadmill and the ground forces were obtained from a similar gait in a nonmoving runway.

The lengths and velocities of each muscle were calculated, including the partial effects attributable to motion at each joint spanned by multiarticular muscles. From the Newtonian equations of motion, an inverse dynamic solution was obtained for the net torque at each joint attributable to muscle tension.

During stance, extensor torque in hip, knee, and ankle peaked together in E2 (40% of stance phase duration), when active extensor muscles were lengthening. Toe extensor torque peaked later in E3, when toe extensor muscles were lengthening, and was associated with a propulsive ground force that cancelled the negative longitudinal ground forces that occurred in the first half of stance.

Swing phase motion involved complex, large angular accelerations and decelerations at all joints, some of which presented real or apparent paradoxes when compared with net joint torques and EMG patterns. Flexion was initiated by synchronous flexor torques at all joints. The transition from flexion to extension in the knee occurred without net muscle torque, being the product of a whipping motion initiated by translational deceleration of the pelvis (during decelerating phase of stance in the contralateral limb). Similarly, the deceleration of hip flexion was attributable to translational acceleration of the pelvis rather than extensor muscle torque. The further angular acceleration of the hip in the extensor direction just before footfall was actually accompanied by net flexion muscle torque, and appeared to be caused by a kickback from the large knee flexion torque applied via the hamstrings to stop knee extension. Whip motion also produced ankle extension, apparently partly opposed by extensor digitorum longus. Deceleration of ankle extension just before footfall occurred despite net extensor muscle torque, suggesting translational accelerations not yet fully understood.

302.10 INHIBITORY PROCESSES POSSIBLY INVOLVED IN MOTONEURON SLOWING DURING SUSTAINED MAXIMUM ISOMETRIC CONTRACTIONS IN MAN. C.G.Kukulka, M.A.Moore* and A.G.Russell. Physical Therapy Research Labs, University of Iowa College of Medicine, Iowa City, IA 52242.

Fatigue of human soleus muscle, induced by sustained maximum isometric contractions, has an associated decline in motor unit firing rates (Neuro.Abs. 10:638,1985). Possible factors responsible for this slowing include peripheral feedback, descending influences recurrent inhibition via Renshaw cell activation, and summation of motoneuron afterhyperpolarizations (AHP). Under nonfatiguing conditions, an H-reflex conditioning technique has been used to indirectly evaluate recurrent inhibition (J.Physiol.269:319,1977). The purpose of this study was to adapt the conditioning technique for use during fatiguing contractions.

Subjects were positioned prone, hip extended, knee flexed 60°, ankle neutral, and foot secured to a torque measuring plate. During initial brief, 2 sec maximum efforts, conditioned (Htest) and unconditioned (Href) H reflexes were obtained. Htest was elicited by a subthreshold motor stimulus followed 15 msec later by a supramaximal stimulus. Single shocks were adjusted to produce Href, of amplitude equal to that of Htest. Both reflexes should be influenced by similar segmental and descending influences. Htest was influenced additionally by the conditioning stimulus. Changes in Htest relative to Href were used to infer the combined effects of recurrent inhibition and summations of motoneuron AHP during sustained efforts.

Subjects performed sustained, 60 sec maximum isometric contractions of triceps surae during which time repeated samplings of Htest and Href were made. Tests were repeated on at least 4 separate days for each subject and changes in Htest and Href were submitted to regression analysis and comparisons made. On average, Htest displayed a depression during the first 30 sec of effort and was highly variable for the remaining 30 sec of effort. Href, on average, displayed an increase in amplitude within the first 30 sec of effort, and was highly variable therafter. The diverging of the two reflexes within the first 30 sec of effort suggests that recurrent inhibition/AHP effects are augmented during this period. These findings provide a possible explanation for the initial slowing of motoneuron firing reported earlier.

This work was supported in part by a grant from the Muscular Dystrophy Association of America

302.11 FUNCTIONAL ROLE OF THE HAMSTER PYRAMIDAL TRACT DURING LOCOMOTION. J. Keifer and K. Kalil. Neurosciences Training Program and Dept. of Anatomy, University of Wisconsin, Madison, WI 53706.

The pyramidal tract (PT) of the hamster projects from the sensorimotor cortex to all contralateral spinal cord segments where it terminates in the medial aspect of lamina V and VI of the dorsal horn. Previous studies on the role of the PT in motor behavior have emphasized the fine movements of the forepaw and digits. We have chosen to examine the function of the PT in locomotion for several reasons. First, locomotion is a centrally programmed, stereotyped behavior in which the coordination of the joints of the limbs can be quantified. Second, locomotion requires accurate placement of the limbs. Finally, defects in this behavior after a lesion of the PT can be accurately measured. Normal freely locomoting hamsters and those with a unilateral section of the medullary pyramid were filmed on two types of terrain: smooth terrain, where sensory feedback is relatively less important for accurate limb placement, and rough terrain, where sensory feedback is more important.

A frame-by-frame movement analysis was conducted by projecting the film onto a screen, tracing the position of the limbs, and measuring the following joint angles: the scapula, shoulder and elbow angle for the forelimb, and the hip, knee and ankle for the hindlimb. When these angles are plotted against time, the coordination of the joints for each limb are revealed. The results show that during normal locomotion, as the forelimb lifts off the ground to initiate swing phase, the scapula and elbow flex, while the shoulder extends. This is followed by a phase of elbow extension midway through swing. When the limb is placed on the ground in stance, the scapula extends and the shoulder flexes. Following a yield in the elbow, a second phase of elbow extension occurs in late stance. Similarly, as the hindlimb lifts off the ground, the hip, knee and ankle flex. Knee and ankle extension occur midway through swing. In stance, the hip extends while the knee and ankle show a second phase of extension. In the lesioned animals, both the first phases of elbow and knee extension in the swing phase drop out so that activation of the elbow and shoulder are completely out of phase, while the hip and knee are in phase. The movement of the joints for a given limb are activated as a unit either in-phase or out-of-phase such that no independent phases of movement can be initiated. In addition, lesioned animals show a deficit in the ability to accurately place the limb while locomoting over the rough terrain. Thus, the results suggest that the pyramidal tract plays an important role in the fractionation of movement and accurate placement of the limbs, both of which are required for normal locomotion.

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302.12 PICROTOXIN AND GLUTAMATE ACTIVATION OF NEURONS IN THE PREOPTIC BASAL FOREBRAIN INITATES LOCOMOTION IN THE ANESTHETIZED RAT. H. M. Sinnamon and M. Kaplan*. Neuropsych. Lab., Wesleyan Univ., Middletown, CT 06457

Locomotion by the anesthetized rat produced by electrical stimulation of the lateral hypothalamus requires projections to the midbrain. Preoptic neurons project axons through the lateral hypothalamus and this study attempted to initiate locomotion by pharmacologically activating their cell bodies.

Male rats were anesthetized with Nembutal and held in a stereotaxic apparatus such that their limbs contacted a wheel which rotated when stepping occurred. A 30-gauge stainless steel canula insulated except for the tip was lowered into the basal forebrain. Cathodal stimulation (0.5-msec pulses, 50-Hz frequency, 10-sec train, <100 uA) was used to test for locomotor effects. Glutamate (20mM or 2M) or picrotoxin (100 or 200 ng) were injected in volumes of 0.2 or 0.1 ul of saline at a rate of 1ul/5min.

Electrical stimulation elicited locomotion in a total of 31 sites which included the lateral (LPO) and medial (MPO) preoptic areas, and the bed nucleus of the stria terminalis (BST). Stimulation in another 23 sites, most in the BST and septal area, failed to produce locomotion. As with stimulation in the lateral hypothalamus and midbrain, the hindlimbs were principally involved in the locomotion.

Stepping was elicited by glutamate in 15 sites; at all of these sites electrical stimulation also produced stepping. Glutamate failed to elicit locomotion at 21 sites. At 6 of these sites electrical stimulation produced stepping, and at 16 it did not. Glutamate locomotor sites were found in the LPO, the MPO and the BST. Longer bouts of locomotion were produced by 2M than by 20nM glutamate.

Picrotoxin injected in 15 sites elicited stepping. At 12 of these sites electrical stimulation was also positive. Picrotoxin was negative in 12 sites, 7 of which were positive for electrical stimulation. The picrotoxin-positive sites were distributed similarly to the glutamate sites but the intensity and duration of the locomotor effects were greater with picrotoxin. The median duration was 10 min and the longest was 30 min.

These results indicate that activity of neurons in the preoptic region can cause locomotor initiation.

302.13 CHANGE IN SPONTANEOUS MOTOR ACTIVITY (SMA) OF RATS TREATED WITH 3-ACETYLPYRIDINE (3-AP), HARMALINE (HLM), AND NICOTINAMIDE (NAA). E. Toyoshima[1], K. Adachi*[2], M. Mukoyama*[2], T. Kihira*[3], K. Ando*[2], and R.F. Mayer[1]. [1]V.A. Med. Ctr. & Dept. of Neurol., Univ. of Maryland Sch. of Med., Baltimore, MD 21201. [2]Natl. Ctr. for Nerv., Ment. & Musc. Dis., Tokyo, JAPAN. [3]Dept. of Neurol., Wakayama Med. Univ., Wakayama, JAPAN.

In this study we compared the open-field SMA of rats treated with 3-AP, HLM, and NAA with 3-AP alone. In group A of 10 rats (8 weeks of age), 75mg/Kg of 3-AP, 15mg/Kg of HLM, and 300mg/Kg of NAA were injected (i.p.) in temporal sequence according to Llinás (1975). In group B of 10 rats, the same dose of 3-AP was injected. 18 rats injected with normal saline were the controls. SMA and locomotion loci were measured and recorded by Animex 2G (LKB-Farad Ltf., Sweden) for 5 min at the same time in a plastic cage (420x 250x400 mm in size). Although only 2 rats in group B survived over 2 weeks, 7 rats in A survived in spite of severe posture and gait disturbances. All rats in A showed wide-base posture and 'mud-walking' within 12 hr, but most rats in B did not show these abnormalities. 1 day after, they showed basically similar signs, and in some long term rats intention-tremor-like movements were observed. SMA (in m, less than 210 mm reflected locomotion) of rats in A, B, and control were as follows (M±SE(No.), *P < 0.01): Before; 13.4± 0.7(5), 13.8±0.8(5), 9.6±1.1(13), 1 day after; 4.8±1.3*(5), 2.4± 0.7*(5), 9.5±1.0(13), 15 days after; 13.2±1.8(7), 8.8±1.0*(2x3), 9.0±1.2(13), 29 days after; 10.4±2.0(5), 13.3±0.7(1x5), 8.8±1.2 (13). Both groups showed significant reduction in SMA of large size (reflected rearing) even after 29 days. Loci of locomotion were similar in both groups. Histological examination showed dark staining neurones without astrocytosis focally in str. pyramidale in the hippocampus in both groups. 2 days after, many degenerated and fewer nuclei with astrocytosis of the inferior olive were observed in both groups. There were also wide-spread degenerated nuclei in the brain-stem in group B. Atrophied and decreased numbers of Purkinje cells with astrocytosis in both vermis and hemisphere were observed in the cerebellum 16 days after in both groups, and this was more prominent later. There were no obvious abnormalities in the rest of CNS and PNS. This study confirmed the observations of Llinás, who suggested that animals treated with these drugs were a good model of chronically degenerated olivo-cerebellar system. Both reduction of SMA and abnormalities in the locomotor loci in the rats treated with 3-AP, HLM, and NAA might occur from destruction of the olivo-cerebellar system rather than the hippocampus. (Supported in part by a grant from the Institute of Health and Welfare, JAPAN and the Veterans Administration, U.S.A.)

302.14 CHANGES IN GLUCOSE METABOLISM IN THE CNS OF THE WALKING CAT. R.J. Schwartzman, E. Eidelberg, and G.M. Alexander*. Department of Neurology, Jefferson Medical College, Philadelphia, PA 19107

The local metabolic rate for glucose (lMRg) was studied in the motor areas of the central nervous system of cats utilizing the quantitative 2-deoxyglucose (2DG) method (Sokoloff, L., J. Neurochem. , 28:897, 1977). Four animals were used as controls and four cats were made to walk on a treadmill (.72 ft/sec). The animals walked for 5 minutes prior to 2DG injection (75 micro Ci/Kg) and during the 45 minute experiment. The lMRg (micromoles/100g/min) was measured in the putative locomotor regions of the midbrain, thalamus, and brainstem. It was also measured in all spinal cord rexed layers, motor cortex, basal ganglia, motor thalamus, cerebellar nuclei, lateral vestibular nucleus, red nucleus, subthalamic nucleus and substantia nigra. The lMRg of all rexed layers in the cervical cord was significantly ($P<0.05$) increased over control values. There was no significant increase in lMRg in the thoracic spinal cord. All layers of the lumbar cord demonstrated greater lMRg compared to control but only rexed layers 1 and 7 were significantly ($P<0.05$) increased. A generalized increase of 10-20% in lMRg was noted in all locomotor regions. The motor cortex demonstrated a 40-70% increase over control values. The caudate, putamen and globus pallidus deomonstrated a 10-20% increase over controls. There was no significant increase in lMRg of the substantia nigra and subthalamic nucleus. The motor thalamus and red nucleus demonstrated a 40-60% increase in lMRg. This technique permits an integrated view of CNS fuction during a specific motor paradigm.

302.15 EXTRACELLULAR RECORDINGS FROM VENTRAL AND DORSAL SPINOCEREBELLAR TRACT NEURONS IN THE SPINAL CORD OF AWAKE, FREELY MOVING CATS. Corey L. Cleland and J.A. Hoffer, Department of Clinical Neurosciences, University of Calgary, Alberta, Canada T2N 4N1.

The dorsal (DSCT) and ventral (VSCT) spinocerebellar tracts are the cerebellum's only monosynaptic sources of information about the hindlimb. Since both tracts, especially the VSCT, receive extensive convergence from descending, interneuronal and sensory pathways, it is difficult to infer their function from experiments in anesthetized or decerebrated cats. Recording the activity of single neurons in the spinal cord of awake, freely moving animals, however, has been impossible because of spinal cord movement. We have solved this problem by using floating microelectrodes to obtain stable extracellular records from VSCT and DSCT axons.

Cats were chronically implanted with: 6-12 microelectrodes in the dorso- and ventrolateral funiculi (L3-T12), bipolar cuffs on the hamstring, sciatic and femoral nerves, stimulating/recording spinal cord electrodes (T2), ankle, knee, and hip angle gauges, and bipolar emg electrodes in 6 hindlimb muscles. Leads converged onto an external 40-pin connector anchored to lumbar vertebrae. Over the next several weeks, signals were recorded and stimuli were delivered via a flexible cable while the cat performed a variety of motor tasks.

VSCT axons were identified by contralateral, short latency group I muscle afferent excitation and an ascending projection. Group I input was evoked by electrical stimulation through the nerve cuffs. The ascending projection was demonstrated by either antidromic activation after spinal cord stimulation or spike-triggered averaging of the spinal cord neurogram. DSCT axons were identified by ipsilateral, short-latency group I afferent excitation and an ascending projection. These criteria excluded other characterized ascending, but not propriospinal, pathways.

We recorded the activity of 90 neurons in four cats. Stability was often sufficient to record from the same axon for 2-12 consecutive days. Thirteen neurons were identified as VSCT (7) or DSCT (6) neurons. During locomotion, five VSCT neurons were active during stance and one was active during swing. The other VSCT axon was most active during light cutaneous stimulation of the anterior hindlimb. The activity of DSCT neurons mirrored the expected activity of muscle receptors in ankle extensor, ankle flexor, toe dorsiflexor and toe plantarflexor muscles.

Our results demonstrate the feasibility of recording the activity of identified neurons in the spinal cord of awake cats during unrestrained movements. In these initial experiments we have begun to characterize the natural activity patterns of spinocerebellar tract neurons in search of their functional roles. (Funded by the Alberta Heritage Foundation for Medical Research)

302.16 LOCALLY INJECTED SUCCINYLCHOLINE ACTIONS ON CAT MULTIJOINT FORELIMB TRAJECTORIES AND THALAMIC NEURONS. V. E. Amassian and L. Eberle*. Dept. of Physiology, SUNY, Downstate Med. Ctr., Brklyn, NY 11203.

The ability of an awake cat to generate a specific multijoint forelimb trajectory, e.g., the initial vertical trajectory of contact placing (CP), raises many questions, including: 1) What are the relative contributions to the trajectory of active (muscle) versus passive forces, e.g., pressure of the dorsum of the paw against the contacted surface, gravity and inertia? 2) Which receptors aid in trajectory regulation? A technique (J. Physiol., 358, 39P, 1985) for injecting succinylcholine (Sch; 30-90 mg/kg) or gallamine (90-300 mg/kg) distally into the brachial artery via an implanted catheter, results in temporary paralysis (or marked paresis) of ipsilateral forearm muscles, as shown by atonia and loss of direct EMG responses to median nerve stimulation via implanted electrodes. The awake cat was not distressed by the injections. Joint angles at shoulder, elbow and wrist-digits were measured at 60 Hz with a TV-computer system. The forelimb trajectory was least affected by gallamine, wrist ventroflexion occurring passively mainly through contact pressure on the paw. By contrast, some CPs by normal cats show increasing wrist ventroflexion when paw pressure is markedly reducing, consistent with activation of forearm flexors as previously shown with EMG recording. Sch effects differed in that transiently, e.g., for 1-2 minutes, angular velocities of posterior flexion at the shoulder and elbow flexion were reduced, while that of wrist ventroflexion increased relatively. At the end of the lifting phase of CP, anterior flexion at the shoulder increased disproportionately to elbow extension, the effect persisting for many minutes. Sch has an additional action of driving strongly Ia spindle afferents (Dutia, M.B., J. Physiol., 304: 315-330, 1980). We tested local Sch on 105 thalamic neurons in or bordering N. VPL contralateral to the injection; they were stereotaxically recorded with tungsten microelectrodes in cats under residual pentobarbital anesthesia supplemented by repeated injections of ketamine(I.M.). Sch activated 36% of neurons, which usually were located anteriorly at the VPL dorsal border and were driven by bending joints or probing muscle below the elbow. Sch inhibited 23%, such neurons usually being driven by , e.g., forelimb skin input or by bending proximal joints.

We conclude that passive forces contribute significantly to ventroflexion of wrist-digits in normal CP; in some CPs, a forearm flexor contribution to the trajectory is readily identifiable. The special effects of Sch probably result from intense Ia driving, e.g.,in the lengthening forearm extensors, which is interpreted ( incorrectly) by the motor control system as an excessive wrist ventroflexion, resulting in reduced central drive to the posterior flexors of the shoulder, which results in high contact pressure on the paw and therefore a marked ventroflexion of the wrist.

302.17 PARADOXICAL SLEEP WITHOUT ATONIA AND CONTROLLED LOCOMOTION: EVIDENCE FOR INVOLVEMENT OF THE SAME SYSTEMS. A.R. Morrison, G. L. Mann,* T. Mitchell,* and G. Cotsarelis.* Sch. of Veterinary Medicine, University of PA, Philadelphia, PA 19104 U.S.A.

Bilateral dorsolateral pontine tegmental lesions eliminate postural atonia and release elaborate behaviors during paradoxical sleep (PS), such as headlifting, righting, "orienting," standing, walking and attack, depending on the exact sites of the lesions (Hendricks, J.C., et al., Brain Res., 239:81-105, 1982). Because cats that walk quadrupedally during PS without atonia have larger, more ventral and more caudal lesions than those that do not, we have suggested that more than one inhibitory system may be damaged by our lesions and that multiple inhibitory systems may be engaged in normal PS. We have now examined whether a region in the dorsal midline of the pons that, when stimulated electrically, inhibits controlled treadmill locomotion induced in acutely decerebrated cats by stimulation of a lateral locomotor strip (Mori, S., et al., J. Neurophysiol., 41:1508-1591, 1978) has a role in PS without atonia.

Surgery for electrode implantations and lesions in 11 cats was performed aseptically under general inhalant anesthesia. Midline electrolytic lesions were aimed for P3-P6 and V -3.5 to -6.0 in cats already displaying PS without atonia as a consequence of bilateral dorsolateral pontine electrolytic lesions placed at least 3 weeks earlier. Waking locomotion was assessed in five 10-minute daily sessions before and after each lesion in a standard open-field chamber. It was significantly increased ($p<0.005$-$0.05$) after the midline lesion in the 5 cats studied. To determine if an additional release from inhibition occurred during PS without atonia after the midline lesion, increases in limb movements (excluding abrupt jerks) made in the first 3 minutes of at least 7 PS without atonia episodes were determined from videotapes. One cat became insomniac and could not be adequately studied in PS. Videotapes of 4 of 7 cats examined in sleep were suitable. They had a statistically significant increase ($p<0.005$-$0.05$) in limb movements during PS without atonia. Two walked in PS after the midline lesion; and 2 already walking increased it significantly. A midline kainic acid injection duplicated the effect of electrolytic lesions in both wakefulness and sleep, but additional chemical lesions with histological analyses are needed to determine with certainty whether cellular or fiber destruction is responsible for the observed behavioral changes.

Thus, the motor inhibition of PS depends on complex brainstem processes and is not solely the province of a circumscribed group of cells in the dorsal pons as has been suggested (Sakai, K., et al., Brain Res., 176:233-254, 1979). We propose that the lateral locomotor strip contributes to locomotor drive in PS without atonia.

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302.18 PERINATAL EXPOSURE TO MORPHINE DISRUPTS POSTURAL SUPPORT IN POST-WEANLING RATS: POSSIBLE FACILITATION OF THE 'IMMOBILITY REFLEX.' Rebecca M. Chesire. Psychology Dept., Univ. Hawaii at Manoa, Honolulu, HI, 96822.*

In some animals, stress or experimentally-induced shock produce akinesia, loss of postural support and loss of righting reflexes; responses that have adaptive value in death feining (Carli, Psy. Rec. 27:Suppl., 1977; Carli et al., Behav. Brain Res. 2, 1981). This form of 'immobility reflex' is believed to be regulated in part by endogenous opiates, and morphine-induced catalepsy resembles the natural defensive state (De Ryck & Teitelbaum, Behav. Neurosci. 98, 1984).

Morphine is a known teratogen that produces persistent (Sonderegger et al., Neurobeh. Toxicol. 1, 1979) and usually deleterious effects in rats. However, this report describes preliminary evidence of a possible facilitation of a component of the immobility reflex in rats exposed in infancy to morphine.

Eighty male and female rats (Long-Evans hooded or Charles River CD albinos) were implanted in the flank on postnatal days 5, 8, or 11 with 75 mg slow release morphine (n=30), lactose placebo (n=30), or no pellets (n=20). On postnatal days 28-30 (5-7 days after weaning), each animal was given one 3 min. trial per day in the open field (a stressful situation).

Rats treated perinatally with morphine showed a significant tendency to lose postural support intermittently during bouts of forward locomotion (other movement differences will be reported elsewhere). The loss of support took three basic forms: (1) complete - no fore- or hindlimb support and the ventrum in contact with the floor, (2) hindlimb - loss of hindlimb support with normal positioning of the forelimbs and (3) mixed - loss of hindlimb support without normal positioning of the forelimbs (e.g., forelimbs splayed beyond the width of the shoulder girdle, forelimbs crossed over one another, or forelimbs leaning markedly to one side). Placebo-implanted and untreated control rats rarely showed a complete loss of postural support, and never lost only hindlimb support or displayed mixed losses. Such support deficits are also absent in rats exposed in infancy to heat, cold or shock stress, or rats injected in infancy with large doses of haloperidol (Chesire, in preparation).

The results suggest that perinatal exposure to morphine may facilitate the development of at least one component that mimics a presumably adaptive death feining response: the loss of complete or partial postural support during stressful stimulation.

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302.19 INFLUENCE OF B-VITAMINS ON THE CONTROL OF FINE MOTORIC MOVEMENTS. D. Bonke and B. Nickel, Messrs. E. Merck, Darmstadt, and Hess. Verband für modernen Fünfkampf, Darmstadt.

The functional role of vitamins of the B-Group in the metabolism of the nervous system is well known by deficiency experiments in animals and volunteers. In case of an avitaminosis the pathologic mechanisms can be described as a blockade of encymatic controlled metabolic steps, in which these vitamins act as coencymes, embedded in the energy-, transmitter- and structural metabolism of nerve cells and also of accompanying cells.

The relevance of functional deficit within the nervous system even due to subclinical deficiency states has been shown in studies by proving cognitive functions or personality structure (J.S. Goodwin et al., JAMA 249:2917, 1983; Chomé et al., Ernährungs-Umschau 91:12, 1984).

It has been stated elsewhere that various transmitter producing encymes (GAD, 5-HTP-D, Dopa-D) are not fully saturated by their cofactor pyridoxal phosphate under normal conditions. The activity of these encymes also seems to be stimulated differentially by pyridoxalphosphate (T.P. Porter and D.L. Martin, J. of Neurochemistry 43:1464, 1984; A.R. Green and D.G. Grahame-Smith, Handbook of Psychopharmacology 3:169, 1975) Thus the question rises, if addition of various vitamin cofactors in dosages above quantities provided by nutritional sources may lead to an improvement of functional nervous system properties.

Because of possible effects as well in sensory as motory pathways the model should involve a performance task in which both aspects may contribute to a performance improvement. The model, by which these conditions are fulfilled, was found in shooting.

The results of two studies (1. open, controlled; 2. double blind versus placebo) with healthy normal marksmen (pentathlon), treated over a period of 8 weeks by a vitamin combination of $B_1$, $B_6$ and $B_{12}$* show that firing accuracy (measured by their succeeded points out of 20 shots and by the deviation of the hits to the centre point of the target disk) improves continuously up to 10 % (1. study $p < 0.05$; 2. study $p < 0.001$). A psychological self-rating test (state trait anxiety inventory) also shows a statistical significant stabilisation of the rating values, which may be interpreted as an increased capability of compensating stress factors, e.g. in competitions, which has been involved in the study.

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303.1 EFFECT OF BICUCULLINE ON SACCADIC EYE MOVEMENT ELICITED BY MICROSTIMULATION OF THE PRE- AND POSTSYNAPTIC COMPONENTS OF FASTIGIAL NEURONS. H. Noda, S. Murakami* and T. Aso*. Visual Sci. Dept., Sch. of Opt., Indiana Univ. Bloomington, IN 47405

Microstimulation of the posterior vermis (lobuli 6 and 7) with electrical currents less than 10 μA elicits a saccadic eye movement in the direction of the stimulation side in the monkey. Similar oculomotor responses can be seen also when the white matter immediately above the fastigial nucleus is stimulated. When the stimulus electrode enters the fastigial nucleus, the direction of eye movements changes from ipsilateral to contralateral side of stimulation. As the synaptic action of Purkinje cells is inhibitory, it is possible that the eye movements in the ipsi- and contralateral directions were caused by stimulation of the pre- and postsynaptic components of fastigial neurons, respectively.

In the present study, oculomotor responses were compared before and after bicuculline injections by using a magnetic search coil technique in three macaque monkeys. The depth of the reversal point of response directions was identified in each track by testing the responses systematically at 100 μm steps. Then, bicuculline (0.2-1 μg) was injected in five loci along the track (1 and 0.5 mm above, 0.5 and 1 mm below, and at the reversal point). Positive effects of bicuculline were observed in 7 out of 8 tracks tested, and the reversal points were histologically identified by iron deposits near the upper border of the fastigial nucleus. Following the injections, the ipsilateral responses from the white matter were almost completely suppressed, while the contralateral responses from the fastigial nucleus were either unchanged or enhanced. The effects continued for several hours but the ipsilateral responses returned to preoperative sizes in less than 10 hours. The negative track was found in the white matter anterior to the fastigial nucleus, suggesting that bicuculline did not reach oculomotor fastigial neurons. It is concluded that the eye movements in the direction of the stimulation side were evoked by activation of the presynaptic component (axons of Purkinje cells from the posterior vermis), while those in the opposite direction resulted from the excitation of the postsynaptic component of the oculomotor fastigial neurons. (Supported by NIH Grant EY-04063).

303.2 ADAPTIVE INTERFERENCE OF OPTOKINETIC EYE MOVEMENT RESPONSE (OKR) WITH VESTIBULO-OCULAR REFLEX (VOR) AND RELATED NEURONAL EVENTS IN THE RABBIT FLOCCULUS. S. Nagao*(SPON:M. Ito). Dept. of Physiol., Fac. of Med., Univ. of Tokyo, Tokyo, 113, Japan

The OKR and VOR operate synergistically to stabilize retinal images of the visual environment during head movement, and have been assumed to linearly interact with each other. However, analyses of the changes induced in OKR under sustained visual-vestibular interaction in cats and monkeys suggest an interaction to take place between adaptive mechanisms of the VOR and those of OKR. In this study on pigmented rabbits, the author found that prolonged optokinetic stimulation, without any vestibular stimulation, induced not only adaptive changes of OKR but also changes in VOR. To locate the source of this adaptive interference of OKR to VOR, effects of floccular lesions and single unit activities of floccular Purkinje cells were investigated. Adaptive changes of OKR were induced in alert pigmented rabbits chronically prepared for fixation on turntable, by sustained sinusoidal rotation (5°, 7.5° or 10° peak-peak; 0.1 or 0.2Hz) of the striped screen. VOR was measured every one hour by rotating the turntable sinusoidally (5° peak-peak; 0.1 or 0.2 Hz) in darkness. During the sustained screen rotation for 4 hours the gain of OKR increased by 0.12-0.26, and at the same time the VOR gain also increased by 0.12-0.22. The VOR gain increased maximally when the screen was rotated by 7.5° peak-peak at 0.1Hz. Slight advancement of the phase (7°-11°) occured in the VOR. In bilaterally floccular lesioned rabbits with local injection of 0.01% kainate, sustained screen rotation no longer produced any noticeable changes in OKR gain, VOR gain or VOR phase. To estimate optokinetic and vestibular responsiveness of floccular Purkinje cells, amplitudes of frequency modulation of simple spike discharges were measured during sinusoidal screen rotation (7.5° peak-peak, 0.1 Hz), and during sinusoidal turntable rotation (5° peak-peak, 0.1 Hz) in darkness, respectively. After the end of recording, brief trains of pulse currents were applied through the microelectrode (tip negative). The floccular areas where the microstimulation induced abduction in the ipsilateral eye were specified as H-zone which was presumed to be involved in the control of horizontal VOR. It was found that sustained screen rotation (7.5° peak-peak, 0.1 Hz) induced significant changes of the optokinetic responsiveness in 75%, and also of the vestibular responsiveness in 57% of the H-zone Purkinje cells tested. The direction of these changes were consistent with the view that these were causal to the changes in the OKR and VOR. Only very minor changes of responsiveness, either optokinetic or vestibular, occured in Purkinje cells recorded from other floccular areas. These observations suggest that OKR and VOR adaptively interfere with each other through their common neuronal circuitry in the flocculus.

303.3 VISUALLY RESPONSIVE NEURONS IN THE FASTIGIAL NUCLEUS OF THE CAT AND ITS MEDIATING PATHWAY. M.Kase*, K.Kawamura, M.Ohno*, T.Hashikawa*, and M.Kato*. Department of Physiology, Hokkaido University, Sapporo 060, Japan (M.K., M.O., M.K.) and Department of Anatomy, Iwate Medical University, Morioka 020, Japan (K.K., T.H.).

It has been said that cerebellar vermis-fastigial nucleus system plays an important role in the control system of pursuit eye movements and optokinetic nystagmus as well as saccades. It remains unsettled, however, whether the fastigial nucleus (FN) receives inputs from the visual system.

Using 11 cats, immobilized by gallamine under urethane anesthesia, 111 FN neurons were extracellularly recorded that responded to electrical stimulation (bipolar: 0.5-0.75 mA 100 μsec) at the optic chiasm. Their responses to the stimulus were classified into three classes: fifty-five FN neurons (type 1) showed a burst, following a transient suppression of discharges. Thirty-seven units (type 2) exhibited a long suppression after a transient burst. Eighteen units (type 3) showed a long suppression associated with the stimulus. In some of our experiments, eight units also responded to a flash of light given to both eyes. Three, four, and one units showed type 1, type 2, and type 3 response patterns, respectively. These classes of units were intermingly located at the dorsal part of the caudal FN where axons of Purkinje cells from the mid-vermal part (lobules VI and VII) of the cerebellum terminate predominantly.

In an attempt to determine the pathways through which the visual information may propagate to FN, HRP was injected iontophoretically into the area of FN where responsive neurons were recorded. In this experiment , a large number of retrogradely labeled cells were found bilaterally in the medial (M) and descending (D) nuclei of the vestibular complex (VN). An injection of HRP in the M and D resulted in the labeling of many cells in the dorsal part of the nucleus reticularis gigantocellularis (Rgc). In another HRP experiment, the nucleus of optic tract (NOT) and the interstitial nucleus of Cajal were shown to give off fibers ipsilaterally to this part of the Rgc.

The present results suggest that the caudal part of FN receives visual information through a channel of the NOT-Rgc-M and D of VN as well as through lobules VI and VII of the vermis where the pontine nuclei send potentially visual information, indicating that the vermis-FN system may participate in the control of optokinetic nystagmus.

303.4 PURKINJE CELL ACTIVITY IN THE FLOCCULUS OF THE ALERT RABBIT DURING NATURAL VISUAL AND VESTIBULAR STIMULATION. PART 2. C. S. Leonard and J.I. Simpson, Dept. Physiol. & Biophys., New York Univ. Med. Ctr., New York, NY 10016.

The simple spike discharge rate of rabbit floccular Purkinje cells (P-cells) was recorded extracellularly during several combinations of vestibular and visual stimulation consisting of velocity step rotations (.1 Hz, ± 2 deg/sec) about the earth vertical axis. As we reported last year, none of the P-cells had the temporal response characteristics of an unprocessed vestibular nerve signal during vestibular stimulation in the dark (VD). Furthermore, one group of cells resembled the 'eye movement only' class of P-cells found in the monkey (c.f. Miles et al., 1980) and, therefore, exhibited virtually no modulation during visual-vestibular conflict (VS) when eye movements were suppressed. We have correlated the discharge rates of these cells with parameters related to both the sensory stimuli and motor responses and here focus on the correlation with eye position. An eye position sensitivity (EPS) was estimated during VD, optokinetic stimulation (OK) and vestibular stimulation with a staircase function. In the rabbit, this latter stimulus moves the eye to different steady positions in the orbit and thereby allows a measure of the steady-state EPS. The mean steady-state EPS was 5.4 spikes/sec/deg (n=4). An apparent EPS was computed during VD and OK stimulation by choosing portions of the stimulus cycle where eye velocity and retinal image velocity were nearly constant and independent of the changing eye position. This apparent EPS was often dependent on the direction of the eye velocity. In the VD condition, the mean apparent EPS was 4.6 spikes/sec/deg for eye velocities ipsilateral to the recording site (the on-direction for all cells) and 2.8 spikes/sec/deg for contralateral eye velocities (n=6). During OK stimulation, this asymmetry was preserved, but the magnitudes were doubled to a mean apparent EPS of 10.8 spikes/sec/deg for ipsilateral eye velocities and 6.1 spikes/sec/deg for contralateral eye velocities (n=6). In summary, the mean steady-state EPS for the rabbit is much larger than that reported for the monkey flocculus (0.7 spikes/sec/deg for 'eye movement only' P-cells; Miles et al., 1980). In addition, the fact that the apparent EPS in the OK condition was roughly twice that in the VD condition suggests that retinal image velocity, which is the adequate stimulus for optokinetic eye movements, manifests itself, in part, as an enhanced eye position sensitivity of floccular P-cells. [Supported by USPHS Grant NS-13742 from NINCDS].

303.5 VISUAL RESPONSES IN "GAZE VELOCITY" PURKINJE CELLS IN THE PRIMATE CEREBELLAR FLOCCULUS. L.S. Stone and S.G. Lisberger, Neurobiology Div., Physiology Dept., UCSF, San Francisco, CA, 94143.

Previous investigators have used sinusoidal stimuli to show that many Purkinje cells in the monkey flocculus have simple spike modulation during visually evoked smooth eye movements. These cells receive vestibular inputs encoding head velocity and oculomotor inputs signalling smooth eye velocity. The vestibular and oculomotor signals sum to produce a firing rate proportional to gaze velocity (eye velocity in the world). There is both anatomical and electrophysiological evidence that the flocculus also receives visual mossy fiber inputs but their influence on gaze velocity Purkinje cells (GVP-cells) is as yet unclear.

We have used a transient visual stimulus to reveal visually driven simple spike responses in GVP-cells related to horizontal eye movement. Cells were identified as GVP-cells according to previous criteria and were then studied while the monkey pursued a step-ramp target movement. This visual stimulus produced 100 msec of 30 deg/sec retinal image motion followed by saccade-free pursuit. For target motion in the ipsilateral direction, the majority of our sample of GVP-cells (21 of 38 cells in 2 monkeys) emitted a clear pulse of simple spikes that was 2 to 8 times larger than could be accounted for by the cell's sensitivity to eye velocity alone. The pulse followed the onset of target motion by about 100 msec, lasted from 75 to 300 msec and coincided with the large eye acceleration during the initiation of pursuit. It was not, however, seen in the responses of eye movement mossy fibers to the same stimuli.

To determine whether the pulse was related to the visual stimulus (retinal slip) or the motor response (eye acceleration), we examined the same cells using a rapid change in head velocity. This vestibular stimulus produced a change in eye velocity comparable to that during step-ramp pursuit but without the preceding visual stimulus. None of the GVP-cells showed a pulse during the rapid eye acceleration evoked by vestibular stimulation, ruling out any significant eye acceleration sensitivity. In addition, GVP-cells with particularly large pulses showed, at the appropriate latency for visual signals (about 100 msec), a difference in their response depending on whether the rapid change in head velocity was imposed in the dark or during fixation of a stationary target. This is caused by the short-lived low velocity image motion at the onset of the rapid change, due to the VOR latency.

We conclude that a large fraction of the GVP-cells in the primate flocculus receive visual mossy fiber input. The strong correlation between the pulse of simple spikes and the eye acceleration suggests that the visual mossy fiber pathways through the flocculus form an important part of the neural command for the initiation of pursuit. (Supported by NSF grant #BNS 8444605).

303.6 DIRECTION OF SACCADIC EYE MOVEMENTS ELICITED BY MICROSTIMULATION OF THE PRE- AND POSTSYNAPTIC COMPONENTS OF FASTIGIAL NEURONS. S. Murakami*, H. Noda, T. Warabi* and T. Aso*. (SPON: A. Strickholm). Visual Sci. Dept., Sch. of Opt., Indiana Univ. Bloomington, IN 47405.

With currents of less than 10 μA, microstimulation of the fastigial nucleus and of the white matter surrounding it elicits a saccadic eye movement. The direction of the eye movement varies depending on whether the presynaptic (vermal Purkinje cell axons) or postsynaptic component (fastigial neurons) is activated. Stimulation of the presynaptic component causes an ipsilateral eye movement, while that of the fastigial neurons causes a contralateral response.

In the present study of three macaque monkeys, the changes in the direction of evoked eye movement in relation to the position of the stimulating electrode were systematically studied, testing at 100 μm steps. Eye movements were recorded with a magnetic search coil technique and analyzed with a PDP-11 computer. When the white matter immediately above the fastigial nucleus was stimulated, the eyes moved to the side of stimulation regardless of the eye position prior to stimulation. However, when the electrode entered the fastigial nucleus, the eyes tended to move toward a point (goal) in the ipsilateral hemifield. Thus, the direction was dependent on the initial eye position. As the electrode was advanced in the fastigial nucleus, the goal gradually moved into the central field and the evoked eye movements were typically "goal-directed". A larger eye movement was elicited when the eyes were far from the goal, and a smaller response was associated with an initial eye position closer to it. Interestingly, the goal moved across the midline into the contralateral hemifield as the electrode passed by the fastigial oculomotor neurons and then moved away to the contralateral periphery. Then, the direction of eye movement was always contralateral. The fastigial loci showing such a goal-shift in the central $30^{o}$ of the field were usually confined to less than 1 mm along a single track. There were, however, variations in the trails of the goal-shift. The goal shifted in some tracks along a straight horizontal line across the primary eye position, while in the other tracks it detoured. In spite of the variations, there was a systematic trend in the type of the goal-shift depending upon the location of electrode tracks in the fastigial nucleus. In 63 out of 85 tracks tested, the goal moved into the central $30^{o}$ of the primary eye position. (Supported by NIH Grant EY-04063).

303.7 ANATOMY AND PHYSIOLOGY OF CEREBELLAR EFFERENTS TO THE VESTIBULAR COMPLEX AND NUCLEUS PREPOSITUS HYPOGLOSSI OF THE SQUIRREL MONKEY. D. B. Belknap and R. A. McCrea. Department of Pharmacological and Physiological Sciences, Univ. of Chicago, Chicago, IL 60637.

The regions of the cerebellar cortex which project to the medial vestibular nucleus (MVN), ventral lateral vestibular nucleus (VLVN) and nucleus prepositus hypoglossi (PPH) were delineated by retrograde transport of WGA-HRP as a part of the physiological and anatomical investigation of floccular efferents which code parameters of horizontal gaze. WGA-HRP was injected iontophoretically and alternate sections were reacted with TMB and left unstained, or with DAB and counterstained with cresyl violet. All retrograde labeling in the cerebellar cortex was ipsilateral to the injection sites. Injection of the central rostral MVN in three monkeys resulted in labeling of a central band of Purkinje cells, extending rostrocaudally through all folia of the ipsilateral flocculus. Labeling of a parasagittal strip of Purkinje cells in the nodulus was also present with MVN injections. A large injection including both the VLVN and MVN resulted in labeling of cells within a broader central floccular band, and scattered labeling in the paraflocculus; the ipsilateral vermis was also labeled in this animal. In two cases in which the injection site included only the lateral portion of the PPH and the adjacent medial MVN, a small region within Crus I was labeled. Labeling of the folium of the paraflocculus adjacent to the flocculus occurred when the lateral PPH was injected. Injections restricted to the medial two-thirds of the PPH resulted in no retrograde labeling of the cerebellar cortex.

In order to determine the orientation of the parallel fibers in the flocculus, particularly relative to the strip of floccular Purkinje cells projecting to the MVN, the parallel fibers were impregnated with silver and their orientation was examined in transverse sections, which were then used to construct a two-dimensional representation of parallel fiber orientation within the floccular cortex. This map was compared with similar maps of the MVN-efferent strip. This analysis demonstrated that the MVN-efferent strip was oriented orthogonally to the parallel fibers.

In order to investigate the physiological properties of the floccular output relating to the control of the horizontal VOR, Purkinje cells coding horizontal gaze are being studied by extracellular recording in alert squirrel monkeys and by injection of an anterograde tracer, Phaseolus vulgaris leucoagglutinin. The preliminary results of this physiological study suggest that Purkinje cells responding to parameters of horizontal gaze may occur in restricted regions of the flocculus.

303.8 CHANGES IN SIMPLE SPIKE ACTIVITY ACCOMPANYING EVOKED CLIMBING FIBER INPUT IN LOCOMOTING CATS. J.H. Kim, J.J. Wang*, and T.J. Ebner. Departments of Neurosurgery and Physiology, Univ. of MN, Mpls., MN 55455

In an accompanying abstract climbing fiber afferent activity was shown to be preferentially associated with a treadmill perturbation which arrested the step cycle. Increases in climbing fiber discharge occurred at the onset of the perturbation and at the resumption of locomotion. This study was designed to examine whether the evoked climbing fiber input was associated with alterations in the simple spike modulation. The presence of concomitant changes in the forelimb muscle activity and displacement was also evaluated. The experimental procedures and recording techniques are described in the accompanying abstract (Wang, et al, Soc. Neurosci. Abstr. 1985). To evaluate the changes in simple spike activity as well as locomotor behavior associated with complex spike occurrence the trials were divided into two groups based on the presence or absence of a complex spike within a time window determined by visual inspection of the complex spike post-stimulus time histogram. The first group consisted of those trials in which a complex spike was evoked within the time window (climbing fiber trials) and the second group those trials in which no complex spike (non-climbing fiber trials) was evoked. From each group of trials a set of histograms (simple spike, complex spike, biceps, triceps, displacement) normalized to the number of trials was constructed. In an additional set of histograms constructed from the climbing fiber trials, the simple spike activity was realigned setting the time of the complex spike occurrence to the first bin in the time window. The biceps and triceps EMG activity as well as limb position were shifted accordingly. Simple spike activity in the climbing fiber trials was quite different from that of non-climbing fiber trials. In the majority of Purkinje cells (16/22) the simple spike modulation increased following the climbing fiber input. In some cells (4/22) the increased simple spike discharge was followed by a reduction in simple spike activity. In two cells the simple spike activity was reduced in the climbing fiber trials. In some cells the increased simple spike discharge was associated with increased triceps activity, and in a few cells the alterations were correlated with the biceps activity. Changes in the step cycle in the climbing fiber trials were also observed. In a walking cat climbing fiber afferent input is associated with changes in the simple spike modulation. These observations are consistent with the hypothesis that the climbing fiber afferent system alters the responsiveness of Purkinje cells to mossy fiber inputs. Supported by NIH grant NS 18338 and NSF grant BNS-8318885.

303.9 CEREBELLAR CLIMBING FIBER AFFERENT ACTIVITY DURING NORMAL AND PERTURBED TREADMILL LOCOMOTION IN THE DECEREBRATE CAT. J.J. Wang*, J.H. Kim, M. Partington* and T.J. Ebner (Spon: F. Torres). Departments of Neurosurgery and Physiology, Univ. of MN, Mpls., MN 55455.

The relationship and contribution of climbing fiber afferent input to volitional or non-volitional motor behavior remains unclear. To begin to answer these questions climbing fiber discharge was evaluated during treadmill locomotion in the cat. A precollicular-premamillary decerebrate cat preparation was chosen because of the spontaneous, treadmill walking these animals exhibit. In these experiments the simple and complex spike discharges of Purkinje cells were examined during spontaneous treadmill walking in which the step cycle could be systematically interrupted. Conventional electrophysiological techniques were used to record and discriminate the simple and complex spike discharge of Purkinje cells in the anterior lobe. Biceps and triceps EMG activity, right forelimb displacement, and treadmill speed were also recorded. Treadmill braking which arrested the cat's locomotion could be timed to occur at specific phases of the step cycle. Peristimulus time histograms of each cell's simple and complex spike discharge, rectified and integrated forelimb EMG, forelimb displacement and treadmill velocity in relation to the step cycle were constructed. In 140 of the Purkinje cells studied the neuron was held for a sufficient length of time to permit averaging its discharge throughout a minimum of 50 step cycles. For 52 cells examined during unperturbed locomotion simple spike discharge was almost always modulated (46/52 cells, 88.4%), usually increasing during the swing phase (23/46). During unperturbed locomotion approximately one third of the cells exhibited complex spike modulation with the step cycle (18/52). Treadmill braking evoked alterations in climbing fiber afferent discharge in 61% of the cells examined (75/122). Different patterns of complex spike discharge were evoked in different cells including increases at the onset of treadmill braking, increases during the period of arrested locomotion and increases at the resumption of locomotion. Reduction in climbing fiber discharge during the period of arrested locomotion was also observed. The most prevalent pattern was an increase in climbing fiber discharge at the resumption of the step cycle (34/75, 48%) following the perturbation period. Complex spike modulation during the unperturbed step cycle suggests this afferent system is active throughout this type of motor activity. The strong association of the climbing fiber afferent discharge to the perturbation suggests a role for this afferent system during changes in motor behavior. Supported by NIH grants NS 18338 and NSF grant BNS-8318885.

303.10 EVOKED PERIODICITY IN CEREBELLAR CLIMBING FIBER AFFERENT DISCHARGE: RELATIONSHIP TO SIMPLE SPIKE ACTIVITY AND RESETTING CHARACTERISTICS. T.J. Ebner and J.H. Kim. Departments of Neurosurgery and Physiology, Univ. of MN, Mpls., MN 55455

Recently the tendency of climbing fiber afferents and inferior olivary neurons to discharge rhythmically and some of the properties of this rhythmic behavior have been described (Bloedel and Ebner, J. Physiol. 352, 1984; Llinas and Yarom, J.Physiol. 315, 1981). In this study additional characteristics of this periodicity and its relationship to simple spike activity were evaluated. Whether the rhythmic discharge was present in a motor behavior was also determined. In the initial part of this study the simple and complex spike discharge of Purkinje cells was recorded in the decerebrate, unanesthetized cat. An ipsilateral forepaw flexion-extension displacement was used to evoke rhythmic complex spike discharge. The resetting characteristics of the evoked oscillation were determined by calculation of the "winding number", a measure of how the evoked rhythmicity interacts with the spontaneous climbing fiber afferent discharge. In most Purkinje cells the complex spike discharge exhibited a winding number of 0 suggesting that the forepaw stimulus completely resets the oscillation. Autocorrelations of the simple spike activity showed that in only a few Purkinje cells was there associated periodicity in the simple spike discharge. Whether the rhythmic climbing fiber afferent discharge occurred in a motor behavior was evaluated in treadmill walking precollicular-premamillary cats. Rhythmicity was evaluated by examining the autocorrelation of the complex spike histogram for specified time periods in the step cycle. Comparable time periods in the triceps and biceps EMG, simple spike discharge and forelimb displacement were evaluated for associated periodicity. Rhythmic discharge of 5-10 Hz was present in the climbing fiber afferent discharge in some Purkinje cells, preferentially in association with an increase in complex spike activity evoked by treadmill braking. Analysis of the forelimb EMG and forelimb displacement revealed little associated rhythmicity . These observations suggest that the forepaw stimulus resets the inferior olivary oscillation which has implications for the underlying mechanisms. Furthermore this rhythmic discharge was observed in a motor activity. In both preparations the rhythmicity was not generally associated with a comparable periodicity in the simple spike activity and in the locomoting animal there was no periodicity in the EMG activity or forelimb displacement, suggesting this oscillation may have a function that is not reflected as a periodicity in the output of the cerebellar cortex. Supported by NIH grant NS 18338 and NSF grant BNS-8318885.

303.11 CROSS-INTERVAL CORRELATION OF FIRING PATTERNS OF SIMULTANEOUSLY RECORDED NEIGHBORING CEREBELLAR PURKINJE CELLS. D.C. Tam, C.K. Knox, and T.J. Ebner. Departments of Neurosurgery and Physiology, Univ. of MN, Mpls., MN 55455

Recently there is renewed interest in simultaneous recording from multiple Purkinje cells. However, analytical techniques to evaluate multi-unit data are limited, relying primarily on cross-correlation analysis. New methods need elucidation. In the present experiments the simultaneous firing patterns of neighboring cerebellar Purkinje cells were examined. Two new analytical techniques are presented for analysis of simultaneously recorded spike trains. These techniques emphasized the temporal relationships among the discharge of the adjacent neurons. Simultaneous extracellular recordings were done on closely spaced Purkinje cells in decerebrate, unanesthetized cats. Poisson distributed pulse trains were used to electrically stimulate forearm nerves and/or parallel fibers to elicit Purkinje cell simple spike modulation without introducing stimulus periodicity. Periodic stimuli were also used at times for comparison. In addition to conventional cross-correlation techniques and post-stimulus time histograms, two new techniques were introduced to analyze the temporal relationship between the simple spike discharge of two neurons. First, the cross-interval histogram was used to assess the relationship between the intervals of the preceding spike of the adjacent cell and the reference cell, and the subsequent intervals between the reference and adjacent cell. In the second technique a cross-interspike interval histogram was used to assess the relationship between the intervals of the preceding spike of the adjacent cell and the subsequent interspike intervals of the reference cell. Both techniques produced a two-dimensional histogram that delineates the effects of a preceding spike of a neighboring cell on the likelihood of subsequent firing intervals. The spike trains were also shuffled, cross-interval correlated, and subtracted from the unshuffled cross-interval histogram to produce the appropriate difference histogram. These techniques permit examination of the conditional probability of the interspike intervals or waiting-time intervals given that a spike occurred in a neighboring cell. The difference cross-interval histograms revealed coupled peaks and valleys present at precise intervals. These preferred intervals of spike occurrence and non-occurrence were not present in conventional one-dimensional correlation techniques. The coupled peaks and valleys profile of these histograms may suggest a temporal surround inhibitory relationship between neighboring Purkinje cells. Supported by NIH grant NS 18338.

303.12 THE RESPONSES OF SIMULTANEOUSLY RECORDED PURKINJE CELLS TO PERTURBATIONS OF THE STEP CYCLE AND THEIR RELATIONSHIP TO THE CLIMBING FIBER INPUT. J.R. Bloedel and J. Lou*. Division of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013.

Experiments were performed in decerebrate ferrets capable of maintained locomotion on a treadmill to examine the responses of Purkinje cells located in various sagittal zones of the cerebellar cortex to perturbations of the locomotor step cycle. The perturbations were produced either by intermittent breaking of the treadmill or the interposition of a rod in the footpath of the forelimb. Up to 6 Purkinje cells were recorded simultaneously in the surface layer of lobules V or VI using an array of 5 tungsten electrodes aligned sagittally and separated by approximately 200 microns. The responses of 37 sets were analyzed using a new analytical procedure, the real time postsynaptic response (RTPR). This method enables a sweep-by-sweep analysis of the combined action of all recorded neurons by calculating a simulated response of a cerebellar nuclear neuron based on the assumption that all the recorded cells terminate on the same postsynaptic cell. The output of a low friction potentiometer attached to the right forelimb was used to trigger the construction of the RTPR and to apply the perturbation at specific and repeatable phases of the step cycle. Each cell's simple and complex spikes were discriminated, and the simple spikes were converted to simulated IPSPs using an alpha function with the appropriate time course. These were then summed linearly to generate the RTPR. The modulation of the RTPR was compared during step cycles in which a perturbation either was or was not applied. Purkinje cells located at one site approximately 1 mm lateral to the paravermal vein characteristically were poorly modulated during the step cycle but responded to the perturbation. These responses were quantified by integrating the RTPR over a specified epoch and comparing this integral in perturbed and unperturbed trials. Across all sets of neurons, the perturbation evoked a 46.59% increase in the modulation of the RTPR as well as a 100% increase in the occurrence of a complex spike in at least 50% of the cells in the set. These data show that sagittally oriented, neighboring Purkinje cells in this cerebellar cortical region respond to the perturbations much more dramatically than to the normal step cycle, and that their responses are associated with the synchronous activation of climbing fiber inputs by the same perturbation. These data are consistent with the hypothesis that perturbations of motor behavior elicit a synchronous discharge of climbing fiber inputs in Purkinje cells located in specific sagittal zones, and that these climbing fibers act to increase the responsiveness of these neurons to mossy fiber inputs activated by the same perturbation. This research was supported by NIH grant #NS-09447.

303.13 THE REAL TIME POSTSYNAPTIC RESPONSE (RTPR): A NEW APPROACH FOR ANALYZING THE RESPONSES OF SEVERAL SIMULTANEOUSLY RECORDED NEURONS AND ITS APPLICATION TO THE CORTICONUCLEAR SYSTEM OF THE CEREBELLUM. J. Lou* and J.R. Bloedel. (SPON: A. Shetter) Division of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013.

Because of the evolving interest in characterizing the response properties of a neuronal population, a method was developed for describing the synaptic action of a simultaneously recorded pool of neurons which have a common site of termination. This method has been applied initially to the corticonuclear system of the cerebellum. Previous methods employed to examine the responses of simultaneously recorded neurons have required either comparison of poststimulus time histograms or various types of cross-correlation techniques. This new approach provides a description of the population's output in terms of its action on a postsynaptic neuron. The first step in the application of this technique requires the conversion of each cell's action potentials to postsynaptic potentials with a specified amplitude and time course. In these initial studies based on the corticonuclear projection, an alpha function was chosen which simulates an IPSP with a time constant of 5 msec. The amplitudes of these potentials were constant and were not corrected for membrane potential. The simulated PSPs were then summed linearly to obtain an analog signal representing the fluctuation in the membrane potential produced by the convergent activity of the recorded nerve cells. One of the most important attributes of this method is that it permits a real time analysis of the action of the recorded neurons without averaging, the construction of histograms, or the calculation of some analytical relationship between the spike trains of the recorded neurons. The spike trains of 3-5 neighboring Purkinje cells are sufficient to generate a RTPR which is sensitive enough to visualize a reproducible response or modulation. The responses can be further quantified by determining the integral of the simulated postsynaptic response for specified epochs related to a stimulus or behavior. One of the most valuable features of this type of analysis is that the effects of the impulse activity of the recorded neurons can be examined on models of postsynaptic neurons with varying complexities. Consequently, the response properties of the postsynaptic neuron can be compared under various hypothetical conditions including various distributions of afferent terminals and variations in the membrane properties. Even under the simplified assumptions used to date, this method has provided a way to characterize the response of a neuronal population based on its action on postsynaptic targets rather than on a comparison between the discharge properties of the presynaptic neurons themselves. This work was supported by NIH research grant #NS-09447.

303.14 LOCATION AND TONIC DISCHARGE PATTERNS OF PHYSIOLOGICALLY-IDENTIFIED PALLIDAL-RECEIVING AND CEREBELLAR-RECEIVING THALAMIC NEURONS IN THE AWAKE MONKEY. R.S. Turner* and M.E. Anderson, Depts. of Physiol. and Biophys. and Rehab. Med. and Regional Primate Research Center, University of Washington SJ-40, Seattle, WA 98195.

Information reaches the cerebral cortex from the globus pallidus and cerebellum via thalamic neurons that seldom receive convergent input from the two sources. Furthermore, it has been proposed from a comparison of anatomical studies that thalamic neurons projecting to the supplementary motor area (SMA) receive input from the pallidum, those projecting to postarcuate area 6 receive input from the posterior cerebellum, and those projecting to primary motor cortex receive input from the anterior cerebellum (Schell and Strick, 1984).

In experiments designed to compare movement-related activity in pallidal-receiving (PR) and cerebellar-receiving (CR) thalamic neurons, stimulating electrodes were positioned in the lateral portion of the ipsilateral internal pallidal segment and in the contralateral brachium conjunctivum (BC). PR-thalamic neurons were inhibited by pallidal stimulation, and CR-thalamic neurons were excited by BC stimulation. The steady-state activity patterns of identified PR and CR thalamic neurons and other nearby cells was determined during a variable 1-3 sec preparatory hold period prior to the visual trigger in a reaction time task. PR neurons had a rather regular tonic discharge pattern, with mean interspike intervals less than 55 msec. CR neurons, however, had lower frequency, irregular discharge patterns with mean intervals greater than 55 msec unless the neuron exhibited particularly high frequency bursts. Recording tracks were run through the entire rostro-caudal extent of VA, VLo, and VPLo, although the pallidal stimulating electrode was not in place during most of the caudal tracks. The recording locations were determined later by reference to electrolytic lesions made in several recording tracks.

Seventy-two hours prior to euthanasia, HRP was injected into the SMA at an A-P position the same as the tip of the arcuate sulcus. Comparison of the location of HRP-labeled neurons and physiologically-identified PR and CR neurons showed that PR neurons were identified in rostral portions of the thalamus, at A-P planes of VA and VLo where HRP-labeled neurons were located. No CR neurons were found in VA or rostral VLo; they were primarily in VPLo, lateral, and at caudal levels, ventral to neurons labeled retrogradely with HRP injected into SMA.

These data are consistent with those of Schell and Strick and indicate that some of the PR thalamic neurons whose activity we have studied probably projected to SMA, whereas the CR thalamic cells sampled physiologically probably projected to the primary motor cortex.

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303.15 PARVOCELLULAR RED NUCLEUS IS IMPORTANT DURING COMPENSATION FOR RUBROSPINAL TRACT LESIONS IN OPERANTLY CONDITIONED RATS. P. R. Kennedy, D. Newby,* M. Wallert,* and D. R. Humphrey. Lab of Neurophysiology, Emory Univ. School of Medicine, Atlanta, GA 30322.

Recordings of histologically verified single parvocellular red nucleus (pRN) neurons in monkeys showed relative unresponsiveness during motor or sensory testing (Kennedy et al., Soc. Neurosci. Abstr. 10[1]:537, 1984). Fiber-damaging electrolytic lesions of the red nucleus in monkeys (Carpenter et al., J. Comp. Neurol. 105:195-249, 1956) failed to ascribe a specific function other than hypokinesia. To determine pRN function, fiber-sparing chemical lesions were made in behaving rats. Since pRN cells partly intermingle with magnocellular red nucleus (mRN) neurons (which project as the rubrospinal tract [RST]), it is impossible to avoid lesioning both pRN and mRN when lesioning the red nucleus. To control for this, bilateral RST lesions were made before nuclear lesions; thus the RST lesions in effect were mRN lesions, and the subsequent nuclear lesions were in effect pRN lesions. Note that RST fibers run in the dorsolateral funiculus and corticospinal fibers run in the dorsal columns, allowing separate lesioning of these systems in the rat.

Long Evans rats (275-450 g) were trained to walk on a 2 cm diameter bar rotating at 9 times per min, 70 cms above a cushioned surface. Rats were averse to falling and so could be operantly conditioned to walk daily for 10 trials, 300 s each trial, with 1 min rest between trials. Naive rats were trained to criterion (250 s average daily trial time) in 3-5 days. Bilateral RST lesions were made in 7 trained rats under Nembutal anesthesia by using needles and fine scissors to sever the dorsolateral funiculus at C3-4. Recovery from quadraparesis occurred in 6-24 h and retraining to criterion in about 1 week (2-10 days). Then 2% Quinolinic acid (Sigma; Schwartz et al., Science 219:316-18, 1983) lesions of each pRN were made about 1 week apart (bilateral same-day lesions were fatal in 2 rats), using corrected stereotaxic coordinates and 1.25-1.50 mm diameter lesions. After unilateral pRN lesions in 3 rats, retraining to criterion occurred in 1-4 days. After subsequent lesioning of the opposite pRN retraining to criterion occurred in 1 day in 1 rat with 98% complete lesions, and was close to criterion in 2 rats before they became ill on day 2 (one had 50% lesions and hydrocephalus; the other is still alive). This suggests that pRN lesions have no effect on rotating bar performance in rats that have already compensated for RST lesions.

To see if pRN has a role to play during compensation for RST lesions, both lesions were performed at the same time by ablating the nucleus with 2% Quinolinic acid (thus destroying pRN plus mRN neurons that give rise to RST). After unilateral lesions in 6 rats, retraining to criterion occurred in a few days. It was possible in 2 rats to lesion subsequently the opposite side. In these rats a prolonged performance deficit occurred. One rat performed at only 20% of criterion for 9 weeks, at which time almost complete lesions were histologically verified (caudal sparing on left). A second rat (with a verified 100% left lesion and rostral 33% right lesion) took over 3 weeks to return to criterion.

These early results suggest that while pRN may not play a role in the control of finely coordinated movement in the already compensated rat performing on the rotating bar, it has an important role to play during compensation for RST lesions. (Supported by NIH grant NS10183.)

303.16 THE ACCESSORY OPTIC SYSTEM IN THE GOLDFISH (CARASSIUS AURATUS): PHYSIOLOGICAL AND ANATOMICAL ASPECTS. J.F. McGurk and W. Graf. The Rockefeller University, New York, NY 10021.

Visual and vestibular responses in the goldfish cerebellum and the pathways mediating them were studied in order to describe the physiological and anatomical context of cerebellar control of eye movements. Field potentials and single unit activity were recorded in the cerebellum following stimulation of the optic nerve and the ampullae of the horizontal semicircular canals. Visually evoked potentials were recorded in a large area of the corpus contralateral to the stimulated eye and in a small region of the ipsilateral corpus along its rostral border. Vestibular potentials were recorded in the center region of the corpus as well as in several portions of the lobus caudalis. Visually evoked field potentials appeared as a positivity on the cerebellar surface and reversed to a negativity at a depth of 1500 μm (maximum amplitude 350-400 μV, latency 15-20 ms). Vestibular field potentials in the corpus exhibited an initial positivity followed by a prolonged negativity. The latency of the positivity was 15-20 ms, the latency of the negativity 40-50 ms. One region in the central one-third of the corpus exhibited activity following stimulation of the contralateral eye and both labyrinths. Into this region of the corpus, horseradish peroxidase (HRP) was injected iontophoretically, after the location had been verified by recording visually-evoked field potentials through the HRP electrode. Large injections of the enzyme into this location produced bilateral retrograde labelling in four pretectal nuclei including the two reported to be the nuclei of the accessory optic system (P1 and P2). In addition, the nucleus lateralis valvulae, the descending and magnocellular octavolateralis nuclei, nuclei in the reticular formation and the inferior olive were labeled bilaterally, as has been described previously. Focal injections into the same cerebellar area, however, produced labelling ipsilaterally only in the pretectal nuclei P1 and P2 of the accessory optic system, in the contralateral inferior olive, and bilaterally in the nucleus lateralis valvulae and the descending and magnocellular octavolateralis nuclei. Intraocular injection of lectin conjugated HRP (WGA-HRP) produced anterograde transsynaptic labelling in both contralateral pretectal nuclei of the accessory optic system (P1 and P2) as well as in cells in the hypothalmus, the dorsomedial optic nucleus and the optic tectum. These physiological and anatomical data indicate that afferents from two sensory systems converge in one area of the cerebellum. This study provides a framework for examining cerebellar control of compensatory and optokinetically evoked eye movements.

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303.17 **EFFECT OF PURKINJE CELL LOSS ON COMPLEX MOTOR BEHAVIOR.** R. Wetts, T. Moran, M. Oster-Granite, and J. Gearhart*. Departments of Pediatrics, Psychiatry, Neuroscience, Cell Biology and Anatomy, and Gynecology and Obstetrics, Johns Hopkins Univ. School of Medicine, Baltimore, MD 21205

The final number of neurons in the mammalian CNS can be reduced by chromosomal aneuploidy, prenatal environmental insults, or genetic mutations. Heterozygous Lurcher (Lc/+) mice lose cerebellar Purkinje cells (PCs) (100%), granule cells (GCs) (90%), and inferior olivary neurons (IONs) (75%) postnatally, but deep cerebellar nuclear cells (DCNs) are spared. In Lc <-> normal aggregation chimeras, all PCs derived from the Lc embryo (10-90%) are lost selectively. All remaining PCs, descended from the normal embryo, are distributed throughout the cerebellum. The reduced number of GCs in the chimera is proportional to the number of PCs that remain. The number of IONs is also reduced in the chimera, but is greater than in Lc/+ animals.

We examined the effects of the reduced number of Purkinje cells by studying selected motor activities in Lc chimeric mice. Lc/+ mice exhibit ataxia, intention tremor, and poor balance. No Lc chimera displays any of these traits. To determine whether subtle, but distinct, alterations occur in the functional capabilities of Lc chimeric mice, we conducted a number of discriminative behavioral tests of cerebellar function on the Lc chimeras, on normal chimeras, and on Lc/+ mice.

All three groups of mice, placed in an automated Omnitech Activity Monitor, were active for similar amounts of time (mean: 20 min/hr) during the night and made similar numbers of movements. Measurement of the number and total time of rearing and mean speed reflected cerebellar function. The mean speed of Lc/+ mice was significantly slower, but that of the Lc chimeras and normals did not differ significantly. The time and number of rearings for Lc/+ mice was also reduced significantly. While these parameters were clearly reduced in the Lc chimeras, the reduction was not significantly different from the normals.

To detect whether the animals displayed hypotonia or poor balance, we measured stride length and width of the hindlimbs. The mice were placed on an inkpad and then allowed to walk across a clean sheet of paper. The results for stride length are, so far, inconclusive. The widths of the Lc/+ animals were greater than in the normals, and the widths of the Lc chimeras were variable, overlapping both the normals and the Lc/+ animals.

Tests of other motor skills are still in progress, but no task examined so far clearly demonstrates any major motor deficit in the Lc chimeric mice. Thus, loss of up to 90% of the PCs in Lc chimeras produces only minor, but distinctive, effects on the functional capabilities of the animal. This supports the idea that there is a large amount of redundancy in the mammalian CNS.

## OCULOMOTOR SYSTEM III

304.1 DEFICITS IN SUPPRESSING SACCADIC EYE MOVEMENTS IN PATIENTS WITH CEREBRAL HEMISPHERIC LESIONS. H.A. Buchtel, R.B. Zuckernik* and S. Berent. Ann Arbor V.A. Medical Center and University of Michigan, Ann Arbor MI 48105

Patients with unilateral frontal lobe excisions for the relief of epilepsy have difficulty looking away from salient visual stimuli presented to the left or right of central fixation (Guitton, Buchtel & Douglas, Exp. Br. Res., in press). We have hypothesized that the frontal eye fields or supplementary motor areas play a role in modulating the activity of lower visual-motor areas which, in the absence of such modulation, may organize maladaptive eye movements to salient visual stimuli. In order to generalize these findings to patients with other lesion etiologies, we have used the same behavioral technique with patients having hemispheric damage from vascular disease, tumor and head trauma.

Two tasks were used. In the first task the patient looked toward a brief visual cue located $10^{o}$ to the left or right of fixation. In the second task (anti-saccade task) the patient had to look away from the cue to a spot in the same position relative to straight ahead but on the opposite side. Eye position was monitored by EOM and recorded for later analysis.

Twenty-one brain-damaged patients (10 left, 11 right) and 12 matched control patients without neurological deficits have been studied to date. Approximately 3/4 of the patients had motor symptoms indicating a disturbance of anterior functions. As in the case of patients after surgery for epilepsy, patients with stroke, tumor and head trauma demonstrated a strong tendency to look toward the visual cue on anti-saccade trials. On approximately 32% ± 7% of anti-saccade trials the patients looked to the wrong side (vs 15%±3% for control subjects). Patients with right-hemispheric lesions made more errors than patients with left-hemispheric lesions (37%±6 vs 25%±7). When the visual fields were considered separately according to the relationship to lesion side, no clear difference between contralateral and ipsilateral fields was seen in group data, although half the patients made at least 10% more errors in one direction than in the other. Lesion site or time since the onset of illness may explain the direction of visual field difference.

These findings extend the observation of a saccade suppression deficit to patients with brain damage from stroke, tumor and head trauma. The presumed mechanism remains the loss of modulatory input from cortical areas to subcortical centers that are capable of organizing saccades to visual stimuli.

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304.2 DEPENDENCE OF THE VESTIBULAR-OCULAR REFLEX GAIN ON RADIUS OF ROTATION AND TARGET POSITION. E. Viirre*, D. Tweed*, K. Milner* and T. Vilis (SPON: J.P. Girvin). Depts. of Ophthalmology and Physiology, University of Western Ontario, London, Canada N6A 5C1.

It has been found in cats (Blakemore and Donaghy, J. Physiol., 300:317-35, 1980) that when the visual target is close to the eyes and the axis of head rotation behind the eyes, there is an increase in the gain of the Vestibular Ocular Reflex (VOR). It can be shown that such an increase in gain is necessary to maintain fixation on the near target.

The precise relation between the horizontal VOR gain and the variables, radius of rotation (R) and target position, was examined in three Macaca fascicularis monkeys. Each had eye search coils implanted and was trained to fixate a LED target during sinusoidal rotation. It was found that the peak-to-peak VOR gain was close to the predicted gain of 1.6 for an object 16 cm from the eyes and with R=10 cm. This gain remained constant for frequencies of rotation from 0.25 to 2.0 Hz. No phase lag was present at a rotational frequency of 2.0 Hz. The increase in gain was also maintained for several cycles after the lights were extinguished. When a sudden rotational acceleration was applied, the increase in gain was apparent within 10-20 ms. These results indicate that the increase in gain must be mediated by the VOR and not by visually driven oculomotor systems such as optokinetic, pursuit or vergence.

The VOR gain was found to increase from 0.94 ± .05 to 1.86 ± .09 with an increase in R from 0 to 10 cm (target distance 11 cm), and to increase from 1.09 ± .03 to 2.02 ± .17 with a decrease in target distance from the eyes from 90 to 8.5 cm (R=10 cm). Throughout a rotation cycle, the instantaneous velocities of the two eyes were not equal differing in a typical pattern which was determined by the fact that the eye that was closest to the target at any time had the higher velocity. It was also found that with eccentric targets the peak-to-peak VOR gains of the eyes could differ by up to 20%. This difference in VOR gain was observed at a rotational frequency of 2.0 Hz and within 20 ms of a sudden head rotation. Thus each eye must have independent VOR gain control.

It is proposed that the VOR gain control is mediated through a combination of semicircular canal and otolith information and depends on an initial determination of the target position with respect to the head.

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304.3 ABDUCTION NYSTAGMUS IN INTERNUCLEAR OPHTHALMOPLEGIA. D.S. Zee and T.C. Hain*, (SPON: D.A. Robinson). Dept. of Neurology, Johns Hopkins Hospital, Baltimore, Maryland 21205

Adduction weakness of one eye and nystagmus on abduction of the other typify internuclear ophthalmoplegia (INO). While interruption of internuclear fibers (VI→III) accounts for the adduction weakness the abduction nystagmus is unexplained. To test the hypothesis that adaptive mechanisms contribute to the abduction nystagmus of INO, we determined the effects of prolonged, monocular viewing (by patching one eye for 3-5 days) on abduction nystagmus in 3 patients with INO. Saccades were quantified by measuring the amplitude of the rapid pulse portion of the saccade (P) and of the final (step) position of the eye (S). The abduction nystagmus was quantified by determining the P-S mismatch (PSM = [P-S]/S) and the time constant of drift ($T_c$).

Patient I had a predominantly right-sided INO but preferentially viewed with the right (paretic) eye. Before patching, for 10° abducting saccades by the left eye (LE), P = 20.7°, PSM = 94% and $T_c$ = 153ms. After 5 days of LE viewing, P decreased to near normal (11.8°) and the abduction nystagmus was markedly attentuated (PSM = 26%.) Likewise, for LE 30° abducting saccades, P decreased from 44.5° to 31.0° and PSM decreased from 52% to 14%. RE adducting saccades showed corresponding decreases in P and increases in PSM. Changes in P and PSM varied with both direction of movement and orbital position. After habitual RE viewing was resumed, saccade metrics and PSM returned to their initial values. Patient II had a bilateral INO with $T_c$ = 94ms. The PSM for LE 10° abducting saccades decreased from 112% after habitual RE viewing to 48% after habitual LE viewing. P also decreased from 24.4° to 17.4°. For RE 10° abducting saccades P appropriately increased (8.8°→14.4°), but PSM changed little (26%→18%). Patient III also had a bilateral INO but with a very rapid postsaccadic drift ($T_c$ = 51ms). After prolonged LE viewing, PSM for LE 10° abducting saccades changed little (85%→66%) and P did not change at all (17.6°→17.7°). For RE 10° abducting saccades PSM did not change (24%→26%) and P increased only slightly (11.75°→13.9°).

Our results indicate that the actions of adaptive mechanisms and which eye is preferentially used for fixation must be considered to interpret the abduction overshoot and abduction nystagmus of INO. In Patient I, in particular, the abduction nystagmus almost disappeared with prolonged viewing by the nonparetic eye. The variability of adaptive affects among different patients with INO may reflect additional lesions that affect structures mediating adaptive control and/or limitations in the adaptive mechanism's capability to suppress extremely rapid and brief postsaccadic drift (e.g., Patient III).

304.4 HYPOTHETICAL MECHANISM OF HEAD-SHAKING NYSTAGMUS (HSN) IN MAN: ASYMMETRICAL VELOCITY STORAGE. Joseph L. Demer. Department of Ophthalmology and Clayton Neurotology Laboratory, Baylor College of Medicine, Houston, Texas. 77030

Head-shaking nystagmus is a jerk nystagmus occurring transiently following a brief period of horizontal head oscillation. This nystagmus is distinct from other forms of pathological nystagmus in that it is not initially present when the head has been at rest for a prolonged period. Clinically, HSN is felt to be a sign of neuro-vestibular imbalance.

HSN was studied in a 21 year old diabetic man who complained of the acute onset several days earlier of vertigo following head movements. After written informed consent, horizontal eye movements were recorded by direct current electro-oculography. No nystagmus was initially present in darkness with the head at rest. Following sinusoidal head rotations in darkness at frequencies from 0.05 to 0.4 Hz lasting 20 to 100 sec (amplitude 60 deg/sec), the head was immobilized but a rightward drift of the eyes developed transiently in each trial. This nystagmus had an initial velocity of 7.5 to 18 deg/sec to the right and decreased in roughly exponential fashion with a time constant of 4 to 13 sec. Vestibulo-ocular reflex (VOR) gain (eye velocity/head velocity) was measured in darkness for 60 to 200 deg/sec steps of head velocity. VOR gain was asymmetrical: 0.62 for slow phases to the left and 0.82 for slow phases to the right. Apparent VOR time constant was 7 sec for slow phases to the left and 10 sec to the right. Although responses to full-field optokinetic stimulation at 50 deg/sec were initially similar, the time constant of optokinetic after-nystagmus (measured in darkness at the conclusion of optokinetic stimulation) was asymmetrical: 1.3 sec to the left and 4.3 sec to the right. Caloric nystagmus evoked by hot and cold irrigations of the auditory canals was bilaterally symmetrical.

These responses were numerically simulated using a mathematical model of the combined vestibulo-optokinetic systems having directional gain and efference copy channels but a single velocity storage element (Demer et al., ARVO Abstracts, 1984, p. 230). In this model, slow phase eye velocity storage is accomplished by a positive-feedback, efference copy loop common to the vestibulo-ocular and optokinetic reflexes. Selection of model parameters matching the observed asymmetrical gain and time constants allowed the model to accurately simulate the HSN observed in the subject. HSN thus appears to be the simply predictable consequence of gain and/or time constant asymmetries in the neural eye velocity storage mechanism. As observed in the subject reported here, the direction of HSN would be predicted to be in the direction of the more effective velocity storage.

304.5 COMPARISON OF SMOOTH PURSUIT AND COMBINED EYE-HEAD TRACKING IN HUMAN SUBJECTS WITH DEFICIENT LABYRINTHINE FUNCTION. R.J. Leigh, J.A. Sharpe, P. J. Rinalli*, M.A. Hamid and S.E. Thurston*. Ocular Motility Lab, Cleveland VA Medical Center and University Hospitals, Cleveland, OH 44106; Playfair Unit, University of Toronto, Ont. Canada, M5T 2S8; Dept. Otolaryngol., Cleveland Clinic, Cleveland, OH 44106.

We studied 8 subjects with deficient labyrinthine function, all with bilaterally absent responses to ice-water caloric stimulation. We measured horizontal gaze (eye position in space) and head rotation using the magnetic search coil technique. Subjects tracked a laser-spot target (a) with eye alone, head still (smooth pursuit-SP) (b) with combined, active movements of eye and head (eye-head-tracking-EHT). The target moved sinusoidally at frequencies of 0.25 Hz or 1.0 Hz with amplitudes of ± 5 or ± 10 degrees. In addition, subjects actively rotated their heads at 0.25 or 1.0 Hz, in darkness, while imagining a stationary target. Subjects also underwent passive, en bloc rotation at 0.25 Hz, 1.0 Hz or lower frequencies, in darkness, while they either imagined a stationary target or performed mental arithmetic. During passive rotation, some subjects were additionally required to look at a head-fixed target ("passive VOR cancellation").

Tracking gain (peak gaze velocity/peak target velocity) was greater during EHT than SP in all subjects for targets moving with a frequency of 1.0 Hz. At 0.25 Hz, EHT gain was greater than SP gain in 4/8 patients. During active head rotation in each subject, the response gain (peak eye velocity in orbit/peak head velocity) was always greater than during en bloc rotation. Nevertheless, during active head shaking, response gain values remained below normal (e.g., at 1 Hz, gains ranged from 0.08 to 0.79). "Passive VOR cancellation" (measured as peak gaze velocity/peak chair velocity) was similar to or greater than values obtained during active EHT.

Although tracking gain was increased in EHT compared with SP, these differences were never large enough to be wholly accounted for by a simple, "linear addition" model in which the smooth pursuit signal cancels the VOR during EHT. In addition, parametric changes or "switches" are required in the model so that transmission of the smooth pursuit signal can be reduced or interrupted during EHT. (Supported by the Veterans Administration, Toronto Western Hospital, MRC of Canada.)

304.6 BRAINSTEM PROJECTIONS OF PRIMATE EXTRAOCULAR MUSCLE AFFERENT NEURONS. John D. Porter. Department of Anatomy, University of Mississippi Medical Center, Jackson, Mississippi 39216.

Although various visual and visuomotor centers (e.g., visual cortex, superior colliculus, cerebellum) receive sensory information from the extraocular muscles, the precise anatomical pathway by which proprioceptive signals are relayed to these centers is unknown. Somata of neurons which provide sensory innervation of primate extraocular muscles are confined to the trigeminal ganglion (J. Comp. Neurol. 218: 208, '83). The present study has utilized the technique of transganglionic transport of wheat germ agglutinin-conjugated horseradish peroxidase (WGA/HRP) from monkey extraocular muscles in order to determine the central synaptic connectivity of these muscle primary afferent neurons.

A cocktail containing WGA/HRP and HRP was injected into various combinations of the four rectus extraocular muscles and monkeys were allowed to survive 24-48 hours. These injections resulted in a discrete pattern of transganglionically-labeled axonal terminals within the ipsilateral trigeminal sensory and main cuneate nuclei. The density of afferent termination varied markedly from animal to animal and from one rostral-caudal level to the next, but was heaviest and observed most consistently within the ventrolateral portion of pars interpolaris of the spinal trigeminal nucleus. A second significant extraocular muscle afferent representation was observed within the ventrolateral segment of the main cuneate nucleus. This projection was restricted to the rostral, reticular, segment of the nucleus, overlapping a region which is known to receive primary afferent input from dorsal neck musculature. Modest to light terminal projections, centered at the border of medullary laminae IV and V, were noted in pars caudalis of the spinal trigeminal nucleus. However, given the variability of this projection, it most likely represents termination sites of periorbital or conjunctival nociceptors. Similarly, light terminal labeling was observed for some cases within the pars oralis and principal trigeminal nuclei.

Taken together, the central termination sites of extraocular muscle afferent neurons are appropriate for relay of proprioceptive information to centers previously implicated in the processing of such information. Beyond serving a simple relay function, target nuclei for extraocular muscle afferents (the main cuneate nucleus in particular) may serve to integrate inputs from eye and neck musculature for the purpose of polysensory coordination of eye/head movements.

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304.7 POINTING PERFORMANCE AFTER STRABISMUS SURGERY IS COMPATIBLE WITH THE OUTFLOW THEORY OF VISUAL LOCALIZATION. O.Bock and G.Kommerell (SPON: W.Daunicht). Dept. Ophtalm. Univ. Freiburg, FRG

Egocentric visual localization of strabismus patients was tested before and after surgical rotation of one eye. If the patients used motor (outflow) signals to determine eye position, localization should change by an amount equal and opposite to surgical eye rotation. If, however, sensory (inflow) information was used, localization should remain unchanged.

Patients pointed without visual feedback of the pointing arm at visual targets, presented in a quasi-random order in 9 horizontal positions within the central ±20 deg of gaze (54 target presentations in total). A mobile, head-fixed device (Ott D, Eckmiller R, Bock O; report in preparation) was used to perform the experiments. Subjects were tested monocularly; repeated tests were performed before, and one about 20 h after surgery, when the operated eye was uncovered for the first time. The amount of surgery was determined from pre-, and postoperative measurements of the squint angle.

On repetition of the preoperative tests, marked changes of the pointing performance were observed in some subjects: we found systematic shifts of the pointing responses by up to 10.8 deg.

After surgery, pointing responses when using the operated eye were shifted opposite to surgical eye rotation in 13 of our 14 patients. On the average, the size of the pointing shift paralleled the amount of surgery, although in some subjects the shift was smaller or larger by up to 12.8 deg. In contrast, pointing responses when using the intact eye remained unchanged after surgery. Findings were similar in patients undergoing their first surgery and in those who had been operated on the same eye muscles before.

The considerable (if compared to normals) ambiguity of preoperative pointing is probably related to the well-known ambiguity of relative visual localization (i.e. to the variability of retinal correspondence) in strabismics. This ambiguity could well explain the differences between amount of surgery and pointing shift, found in some subjects. Further, the one subject without pointing shift might have used subtle cognitive cues (e.g. recollection of preoperative target positions) to properly adjust an ambiguous localization after surgery; as professional sharpshooter, he probably used such cues more effectively than others.

Steinbach and Smith (Science, 213:1407,1981) found only small pointing shifts after strabismus surgery in the majority of their patients; it is conceivable that their testing procedure facilitated use of cognitive cues, e.g. by using only 3 target positions.

We conclude that the outflow theory of visual localization, supported by a variety of findings from different paradigms, is also compatible with the pointing performance after strabismus surgery, once the well-known ambiguity of visual localization in strabismics is taken into account. Supported by Dt.Forschgs.gem.

304.8 MULTIPLY INNERVATED FIBERS IN MAMMALIAN EXTRAOCULAR MUSCLE WITH VARIATION ALONG THEIR LENGTH OF THEIR ELECTRICAL ACTIVITY AND MYOSIN CONTENT J.Jacoby, E.Stefani*, J.Davidowitz, G.Philips and D.J. Chiarandini, Depts. Ophth. and Physiol. and Biophys., N.Y.U. Med. Center, New York, NY 10016 and *Dept. of Physiol., Center for Adv. Studies, I.P.N., Mexico D.F. 14.

Mammalian extraocular muscle, unlike most other mammalian muscle consists of multiply innervated (MIF) and singly innervated (SIF) fibers which are intermingled in two morphologically distinct layers: the global and orbital. Global MIFs generate tonic tension and lack action potentials. On nerve stimulation they give endplate potentials (epp's) and graded, slow voltage dependent responses or slow peak potentials. The contractile properties of orbital MIFs are unknown. Unlike the global MIFs, which exhibit a moderate to poor development of sarcoplasmic reticulum (SR) along their length, the morphological properties of orbital MIFs are complex. Toward their middle the fibers have a very small diameter (5-7 um) and they display well developed SR with small myofibrils (Fibrillenstruktur) and a twitch-like endplate, coextensive with the orbital endplate zone (EPZ) of the muscle. Distally and proximally to the EPZ, the diameter typically increases gradually to 10-15 um, the myofibrils become larger and progressively less defined, approaching an afibrillar morphology (Felderstruktur) at both ends. Superficial nerve endings are scattered in these regions. A monoclonal antibody against chick ALD muscle binds in the Felderstruktur regions but not in the Fibrillenstruktur portion of the fiber. We studied "in vitro" the electrophysiological properties of orbital MIFs in inferior rectus muscle of the rat. We found that orbital MIFs are innervated by multiple axons and that on nerve stimulation they produce a complex response that varies along the length of the fiber. Near the EPZ distally to 2 mm, nerve stimulation produced compound epp's at low stimulus strength and a non-overshooting, fast-rising voltage-dependent response or "spike" at higher stimulus strength. Distal to 2 mm the "spikes" were absent or greatly attenuated. The responses in most cases consisted of large and small epp's, similar to those found in global MIFs. Lucifer yellow injections of fibers identified electrophysiologically as orbital MIFs filled the entire length of the fiber from the distal end to the EPZ, spanning regions of different morphology and electrical activity, supporting morphological evidence of a single continuous fiber. We hypothesize that the apparent dual nature of these fibers along their length is maintained through a dual innervation by a fast motoneuron and one or more slow motoneurons. Supported by USPHS EY01297, EY07009, EY00309, NSF INT 7920212, and CONACyT 790022.

304.9 PONTINE RETICULO-SPINAL NEURONS ARE A COMPONENT OF RETICULAR CIRCUITS CONTROLLING EYE-HEAD SYNERGIES. A.Grantyn[x], A.Berthoz and V.Ong-Meang[x], Lab. de Physiol. Neurosensorielle, CNRS, Paris,France

During orienting towards eccentric targets, eye and head movements are often synergic. It has been suggested (Vidal, P.P. et al, Exp.Brain Res.,46:448, 1982) that pontine reticular neurons may participate simultaneously in the control of these two motor components of orienting. To provide direct evidence for this supposition, we studied behavioral properties of identified pontine reticulo-spinal neurons (PRSNs) considered, on morphological grounds, as candidates for mediating eye-head synergies. Experiments were performed on alert, untrained, head-fixed cats. Orienting responses were elicited by presentation of moving visual stimuli. Axons of PRSNs were penetrated intracellularly close to the abducens nucl. and identified by antidromic responses to ipsilateral spinal cord stimulation (C1, C2) and monosynaptic activation from the contralateral superior colliculus. Two axons were injected with HRP, to visualize their branching pattern in the brainstem. Relationships between neuronal activity and motor events during orienting (eye movements, EMG-activity of dorsal neck muscles) were analysed quantitatively.

Four out of 14 PRSNs did not show any clear relation to eye movement or neck EMG. The activity of the remaining 10 PRSNs was qualitatively similar during horizontal eye-head synergies : 1)Absence of any discharge when eyes are deviated in contralateral direction, 2) Phasic activity showing about the same degree of correlation with ipsiversive horizontal saccades and phasic components of neck EMG, 3) Tonic activity roughly proportional to ipsilateral horizontal eye position but not maintained during prolonged eccentric fixations, 4) Variability of the degree of correlation between discharge profiles and motor events. During dissociation of eye-head synergy, PRSN activity may be related to neck EMG while eye position does not change. The two HRP-labelled neurons displayed discharge patterns described above. Their somata were located rostroventrally to the abducens nucleus and collateral branching in the lower brainstem corresponded to the type characterized by R.Grantyn et al. (Brain Res., 198:221, 1980). Target areas included the abducens and facial nuclei, the vestibular nuclei, the prepositus/intercalatus complex and the caudal pontine and bulbar RF. Morphology of terminal structures presented features of both diffuseness and specificity. Of particular interest were the cases of dense perisomatic clusters containing up to 27 boutons derived from convergent collateral branches of second and higher orders. PRSNs with extensively branched axonal tree appear thus to establish selective connections with particular target neurons.

In conclusion, PRSNs of the periabducens area which project to spinal cord, abducens and facial nuclei, and bulbar sites of origin of parallel reticulo-spinal pathways do generate signals adequate for partial execution of horizontal eye-head synergies.

304.10 PHYSIOLOGICAL CHARACTERISTICS OF NEURONS IN THE MEDIAL VESTIBULAR NUCLEUS AND RETICULAR FORMATION OF THE SQUIRREL MONKEY INVOLVED IN FOVEAL CANCELLATION OF THE HORIZONTAL VESTIBULO-OCULAR REFLEX. Eugene F. May* and Robert A. McCrea. Committee on Neurobiology, Dept. of Pharmacol. & Physiol. Sci., University of Chicago, Chicago, IL 60637.

The rostral part of the vestibular complex and adjacent reticular formation were explored for neurons which carried signals related to foveal cancellation (FC) of the horizontal vestibulo-ocular reflex (VOR). Squirrel monkeys were trained to fixate a small light attached to a vestibular turntable on which they were rotated sinusoidally in the yaw axis. Three groups of neurons were found whose firing rate was related to FC of the VOR, and their response during vestibular and optokinetic nystagmus (OKN), smooth pursuit, and FC of the VOR and OKN was studied.

In the rostral-most part of the medial vestibular n. (MVN) and subjacent reticular formation, cells were found whose head velocity sensitivity increased without changing phase when the monkey cancelled its VOR. The enhancement was most pronounced at higher stimulus frequencies and velocities. The firing rate of these cells was not correlated with other types of eye movement.

In the dorsomedial part of the MVN at the level of the caudal abducens n. and n. prepositus, a second group of neurons was identified whose firing rate was related to ipsilateral eye position, ipsilateral eye velocity (except during OKN) and ipsilateral head velocity. During FC of the VOR, the neurons' firing rate lead ipsilateral head velocity by $5^0$ to $20^0$. The cells' head velocity sensitivity was not apparent at velocities less than $10^0$/s during VOR cancellation, and there was no apparent head velocity or eye velocity sensitivity when the VOR was not cancelled. During OKN and FC of OKN, the firing rate of these neurons was related only to eye position and saccadic eye velocity. These cells may participate in the generation of saccadic and smooth pursuit eye movements, and in cancelling at the level of the motoneuron the vestibular signals carried by secondary VOR neurons during VOR cancellation.

The third group of cells, similar to those described by Eckmiller and Bauswein in the rhesus monkey (Soc. Neurosci Abs., 10: 391, 1984), was located just ventral to the rostral medial vestibular nucleus, adjacent to the ventral border of the abducens nucleus. These cells fired only when contralateral slow phase eye velocities induced by head movements or peripheral retinal slip were suppressed, which suggests that they may play an important role in this ability. They did not fire during spontaneous eye movements except during ipsilateral smooth pursuit. During sinusoidal head rotation, they fired only when the VOR gain was less than 1.0. During FC of the VOR, the firing rate of the cells lead ipsilateral head velocity by $0^0$ to $20^0$; they were sensitive to head velocities as low as $2^0$/s and the gain and phase of the response were constant from 0.1 Hz to 1.0 Hz. In addition, the head velocity response was superimposed on a tonic firing rate whose onset immediately preceded the act of FC and which lasted as long as the FC continued. The amplitude of this component of the signal appeared to be related to the frequency of sinusoidal head rotation. During OKN, the neurons fired only when slow-phase eye velocity did not match the optokinetic stimulus velocity. During FC of OKN, their firing rate was linearly related to the slow phase eye velocity being suppressed; higher firing rates were associated with suppressed slow phase velocities in the contralateral direction.

These data suggest that foveal cancellation of vestibular nystagmus and optokinetic nystagmus may be mediated by different premotor pathways.

304.11 **TRANSSYNAPTIC RETROGRADE STUDIES OF THE NUCLEUS OF EDINGER-WESTPHAL AND THE OCULOMOTOR SYSTEM.** Jonathan T. Erichsen and Craig Evinger. Dept. of Neurobiology and Behavior, SUNY at Stony Brook, NY 11794.

The avian ciliary ganglion contains two subpopulations of cells: the ciliary, which project to the iris and the ciliary body, and the choroid, which project to the choriocapillaris. Neurons of the nucleus of Edinger-Westphal (EW) provide the only known input to the ciliary ganglion, and studies of the afferent projections of EW indicate that it consists of at least three distinct subdivisions. In the present study, we used transsynaptic retrograde transport to determine the peripheral motor targets of subdivisions of EW.

Wheat germ agglutinin (WGA) (8ul at 10ug/ul) was injected into either the iris or the ciliary body. After 24h, WGA (using immunohistochemistry) was found in the cells of the ciliary ganglion but in no central structures. After 3 to 6 days survival, specific labelling was found in one or more subdivisions of EW, as well as in cells of the ciliary ganglion. Control HRP injections into these two target sites always labelled cells of the ciliary ganglion but never within EW. Thus, we conclude that the retrograde label found in EW results from transsynaptic retrograde transport of WGA via the cells of the ciliary ganglion rather than a direct projection from EW to the target sites.

Injections to the iris labelled neurons in the extreme lateral portion of EW throughout its rostrocaudal extent as well as the entire caudolateral subdivision. The location of these labelled cells corresponds to the afferent termination from the pupilloconstrictor pretectal nucleus, area pretectalis (Gamlin et al., 1984). In contrast, injections to the ciliary body also labelled neurons in the rostrolateral subdivision, suggesting that this subdivision mediates the control of visual accommodation.

In addition, injections into the anterior chamber (i.e., iris injections) resulted in distinct terminal labelling within the various sensory trigeminal nuclei that corresponds to projections of the ophthalmic branch of the trigeminal nerve (Dubbeldam & Karten, 1978). We attribute this to anterograde transperikaryal transport through the Gasserian ganglion via the ophthalmic branch. Labelling of cells in the ipsilateral accessory abducens nucleus suggested transsynaptic anterograde transport. Unilateral injections of WGA into extraocular muscles retrogradely labelled the expected oculomotor neurons, but also resulted in transsynaptic labelling of contralateral oculomotor neurons.

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304.12 CILIARY GANGLION AFFERENT NEURONS: LOCATION AND CHOLINE ACETYLTRANSFERASE STAINING IN THE CAT. L. Brezina*, A. Strassman*, P. Mason, F. Eckenstein, and R. Maciewicz, Pain Physiology Lab, Neurology Service and Neuroscience Program, Mass. General Hospital and Harvard Med. School, Boston MA 02114.

Ciliary ganglion afferents are cholinergic neurons traditionally thought to reside within the Edinger-Westphal nucleus (EW). However, in the cat ciliary ganglion afferents are located primarily outside EW; EW neurons in contrast have extensive projections to spinal cord and cerebellum. In the present study the distribution of preganglionic parasympathetic neurons that project to the ciliary ganglion was compared to the pattern of cells showing choline acetyltransferase (ChAT) immunoreactivity in the midbrain.

Following horseradish peroxidase (HRP) injections into ciliary ganglion, labeled preganglionic neurons were found largely outside EW, scattered in the ventral periaqueductal gray region capping the oculomotor nucleus (nIII) as well as in the tegmentum ventral and anterior to nIII. Some preganglionic neurons were also observed in the ventral part of the anteromedian nucleus. Midbrain sections processed for ChAT immunohistochemistry revealed a pattern of ChAT- positive neurons outside of nIII that closely parallelled the distribution of preganglionic cells. In contrast, cells showing substance P- or cholecystokinin- like immunoreactivity were found primarily within EW. Using a two-chromogen method (3,3'-diaminobenzidine (DAB) and alpha-napthol/pyronin B or cobalt-DAB and DAB) ciliary ganglion afferent neurons identified by retrograde HRP transport also showed ChAT immunoreactivity.

To determine whether ciliary ganglion neurons have intracranial projections, in the same experiments nuclear yellow was injected into either the deep cerebellar nuclei or the cervical spinal cord. In both cases cells labeled with fluorochrome were found throughout the length of EW; however, there was little overlap of the distribution of ciliary ganglion afferents with the distribution of cells projecting to either cerebellum or spinal cord. No preganglionic neurons were labeled by either cerebellar or spinal injections.

These findings provide further evidence that in the cat ciliary ganglion afferent neurons differ in their immunocytochemistry, location, and projections from cells in EW.

304.13 DYNAMIC DIFFERENCES BETWEEN AIRPUFF- AND LIGHT-ELICITED EYEBLINKS. K.A. Manning* & C. Evinger. (SPON: L.A. Riggs). Dept. Neurobiol. & Behavior, SUNY at Stony Brook, Stony Brook, NY 11794.

An eyeblink is a commonly occurring and rapidly accomplished oculomotor activity produced in response to many stimuli. Nevertheless, the neural basis of blinking is not well understood. As a first step towards examining the neural control of blinks, we measured the dynamics of airpuff-elicited (A-) and light-elicited (L-) blinks in unanesthetized rabbits. Specifically, we wanted to find out whether these reflex blinks were similar, except for expected differences in blink latency, or if, in fact, the dynamics of blinks differed depending upon the sensory pathway that produced them.

Lid movement was measured with a photosensitive device activated by a light emitting diode at the end of a light-weight lever arm that was attached to the upper lid margin. This arrangement did not impede lid movement. Blink-related lid position and velocity data produced in response to air and light stimuli of varied duration (25-700ms) and strength were recorded. In several rabbits, the levator palpebrae (LP) and orbicularis oculis (OO) EMGs were also recorded simultaneously. We found that the dynamics of the L- and A-blinks were consistently different, even when blink amplitudes were equal. During the initial downward movement of the lid, maximum lid velocity and acceleration were typically twice as great for A-blinks as for L-blinks. Also, once the change in lid position had begun, time to maximum lid velocity occurred twice as quickly in A-blinks as in L-blinks. The EMG showed the expected reciprocal relation between LP and OO activity for all blinks, but the timing of activation of OO with respect to cessation of LP varied. Cessation of LP activity usually preceded activation of OO during A-blinks, but occurred near the time of, or after, excitation of OO during L-blinks. Unexpectedly, LP exhibited a weak period of activation before cessation of activity during L-blinks. The pattern of OO activity also differed with A- and L-blinks. Airpuff-evoked OO activity began with a burst, while light-evoked OO activity increased gradually and continued for longer durations.

These results indicate that, in the rabbit, different afferent pathways produce blinks with different dynamics. All blinks do not appear to be generated by the same neural circuit. Instead, blinks may be produced differently by various afferent sources. We are repeating these experiments with human subjects.

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304.14 ADAPTIVE PLASTICITY OF REFLEX BLINKS. C. Evinger and K.A. Manning*. Dept. Neurobiology & Behavior, SUNY Stony Brook, Stony Brook, NY 11794

Understanding adaptive plasticity requires developing model systems that are advantageous for physiological and anatomical study. Because of its relative simplicity, the reflex blink of the rabbit offers such a model system. Movements of the upper eyelid involve essentially two muscles: orbicularis oculis to pull the eyelid down and levator palpebrae to pull the eyelid up. Reflex blinks can be elicited by two different, presumed three neuron pathways: trigeminal and visual. We now report that adaptive changes occur in trigeminally evoked blinks in less than 1 hour. Thus, reflex blinks in the rabbit are a simple, mammalian system for neurophysiological investigation of adaptive plasticity.

Before, during, and after lid adaptation, reflex blinks of a moderate size were evoked in unanesthetized rabbits with a weak 50ms airpuff or a 200ms flash of light directed at the eye. These stimuli occurred at a mean interstimulus interval of 10 sec. Position of the upper eyelid and orbicularis oculis EMG were continuously monitored. Experiments began with 30 air puff trials to collect baseline data. To induce adaptation, the upper eyelid experienced a constant, upward tension produced by attaching a 9gm weight to the upper eyelid via a pulley. After attaching the weight, the rabbit underwent 240 airpuff trials over a period of 55min. Sixty airpuff trials followed removal of the weight. In the preweight trials, the airpuff stimulus evoked blinks with a mean amplitude of 1.2mm and a mean acceleration of 357 mm/sec$^2$. The mean amplitude and acceleration of blinks upon removal of the weight rose to 2.3mm and 1508mm/sec$^2$. The increased (422%) intensity of reflex blinks following weight adaptation slowly decayed, so that 40-60 trials after removal of the weight, blinks evoked by airpuffs were back to baseline levels. Changes in the orbicularis oculis EMG mirrored the changes in eyelid dynamics. Light elicited blinks showed no evidence of adaptation, suggesting that the adaptation was modality specific.

Thus, a short period of adaptation to upward tension on the upper eyelid produced an increase in the amplitude and speed of the downward movement of the upper eyelid with a trigeminally evoked blink. Given the relatively simple organization of the blink reflex, this should prove a fruitful preparation for studying the neural control of adaptive plasticity.

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304.15 CEREBELLAR CONTROL OF THE CONDITIONED NICTITATING MEMBRANE RESPONSE IN RABBIT: BILATERAL NEURAL PLASTICITY. B.E. Polenchar and M.M. Patterson, Department of Psychology and College of Osteopathic Medicine, Ohio University, Athens, OH 45701.

Recent studies conducted by R.F. Thompson and colleagues (McCormick & Thompson, Neuro.Abst., 1983, 9: 643; McCormick et al., Bull.Psych.Soc., 1981, 18: 103;) have implicated the cerebellar nuclei as an essential component of the memory system for the conditioned nictitating membrane (NM) response in rabbit. Unilateral lesions of the cerebellar deep nuclei abolish the learned NM response in the eye ipsilateral to the lesion, but do not affect the reflex response, or the animal's ability to acquire conditioned responses (CRs) when training is shifted to the contralateral eye. In replicating these effects (Polenchar & Patterson, Neuro.Abst., 1984, 10: 123.), we noted that when training was shifted to the opposite eye after nuclear lesions, there was a significant reduction in the number of trials taken to reach criterion. The present study investigated the possibility that a certain degree of bilateral neural plasticity occurs during initial acquisition (McCormick & Thompson, 1981). In the first experiment, we simply replicated the McCormick and Thompson procedure, while monitoring the responses of both eyes throughout all phases of training. We found that although CRs were acquired concurrently in both eyes during initial acquisition, unilateral cerebellar nuclei lesions tended to abolish CRs in both eyes. However, after the contralateral eye was trained to produce CRs, responding in this eye persisted even after training was shifted back to the ipsilateral eye. In addition, there is often a small amount of ipsilateral recovery after contralateral training. This pattern of results may suggest that initial responses of the untrained eye result from bilateral control of the conditioned NM response by a unilateral cerebellar system. In the second experiment, a multiple unit recording electrode was chronically implanted in the dentate/interpositus (DI) region on one side of the cerebellum and lesioning electrodes were implanted on the other side. The same sequence of pre- and post-operative training was used, while multiple unit recordings were made from the intact nuclei during all stages. While only preliminary results are available at this time, there is strong evidence to suggest that neural unit activity related to the learned response of the trained eye occurs in the contralateral cerebellum, similar to that found in the ipsilateral cerebellum (c.f. McCormick, et al., 1981). In some cases, we found that an increase in ipsilateral DI unit activity occurred in the absence of a response in the untrained (ipsilateral) eye. The pattern of results found thus far indicates that activity of the ipsilateral cerebellum may be projected to the contralateral hemisphere (consistent with McCormick an Thompson's prediction), but does not necessarily produce a response in the contralateral eye.

304.16 PROPERTIES OF VESTIBULAR INPUTS TO THE SITE OF ADAPTIVE CHANGES IN THE VESTIBULO-OCULAR REFLEX (VOR) IN MONKEYS S.G. Lisberger and T.A. Pavelko*, Div. Neurobiology and Dept. Physiology, UCSF, San Francisco, CA.

The accuracy of the VOR is regulated by a long-term adaptive mechanism that is used whenever the VOR is in error. The behavioral adaptations are well understood, but the modifiable neural pathways subserving adaptation remain unidentified. We have used a transient vestibular stimulus in two experiments to define the modifiable pathways according to the response properties and latency of the vestibular inputs they transmit.

First, we used "rapid changes in head velocity" to study the VOR in the dark before and after adaptation to magnifying and miniaturizing spectacles. The vestibular stimulus provided head acceleration from zero to 30°/s in 50 ms, followed by constant head velocity at 30°/s for 300 ms. The sudden head acceleration allowed us to measure the transient response of the VOR (latency and degree of overshoot). The constant head velocity allowed us to estimate the steady-state gain of the VOR (eye velocity divided by head velocity). The transient response changed as a function of steady-state gain. Normally, eye velocity showed slight overshoot so that peak eye velocity was 1.2 times steady-state. When steady-state VOR gain had decreased to 0.3, the transient overshoot was accentuated so that peak eye velocity was 2 times steady-state. When steady-state gain had increased to 1.8, there was no overshoot. Changes in VOR gain did not affect the latency or the first 5 msec of the VOR.

Second, we recorded the activity of 73 vestibular nerve fibers innervating the horizontal canal in 2 normal monkeys. We studied the response to rapid changes in head velocity by averaging instantaneous firing rate for 50-100 consecutive stimuli. Each fiber was then characterized by the degree of overshoot in firing rate and the response latency, as well as by the regularity of discharge with the head still. Afferents with the most regular discharge responded at latencies of 8-18 ms and had little or no overshoot. More irregular afferents responded at latencies of 5-10.5 ms and had substantial overshoot in firing; the peak change in firing rate was 2-6 times as large as the steady-state change.

Our eye velocity data can be accounted for by our nerve recordings if the modifiable VOR pathways receive inputs from afferents with the most regular discharge. In addition, the absence of changes in the first 5 msec of the VOR and the large transient response when steady-state gain is low imply that some pathways cannot be modified. These pathways would receive inputs from more irregular afferents, which have shorter latencies and larger transient responses. (Supported by NIH grant EY 03878.)

304.17 A MODEL FOR TARGET-DEPENDENT GAIN CONTROL IN THE VOR. D. Tweed*, E. Viirre* and T. Vilis. Depts. Ophthalmology and Physiology, University of Western Ontario, London, Canada, N6A 5C1.

Ideal vestibuloocular gain, the ratio of eye rotational velocity to head rotational velocity which will minimize retinal image blur, depends on the location of the visual target and on the linear and angular motion of the head. The ideal gain need not equal -1, it may be different for the two eyes, and it must be continuously modulated according to target location relative to the moving head. Experiments in this laboratory have shown that the gain of the VOR in monkeys has all these properties and closely matches that of an ideal VOR (see accompanying abstract Viirre et al.).

To account for the target-dependent flexibility of VOR gain, we propose a model in which the gain of the vestibuloocular pathway is governed by a target locator which uses vestibular signals to compute the craniotopic motion imparted to the target by rotation and translation of the head. If $\dot{\mathbf{x}}$ is the craniotopic velocity of the target, the formula for its computation based on a head rotational velocity **R** signal from the semicircular canals and a head translational velocity **T** signal derived from otolith organ output is

$$\dot{\mathbf{x}} = (\mathbf{x} \times \mathbf{R}) - \mathbf{T}.$$

The vector **x** is craniotopic target location, given initially by visual, auditory or other sensory input and updated by integrating $\dot{\mathbf{x}}$. For each eye, the desired gaze vector **g** is defined as $\mathbf{g} = \mathbf{x} - \mathbf{e}$, where **e** is the fixed craniotopic location of the centre of that eye. The vectors **g** and $\dot{\mathbf{g}}$ for each eye are readily obtainable from the output of the locator, and they in turn yield $\boldsymbol{\omega}$, the desired angular velocity of the eye, by the equation

$$\boldsymbol{\omega} = (\mathbf{g} \times \dot{\mathbf{g}}) / |\mathbf{g}|^2 - \text{proj}_{\mathbf{g}}\mathbf{R}.$$

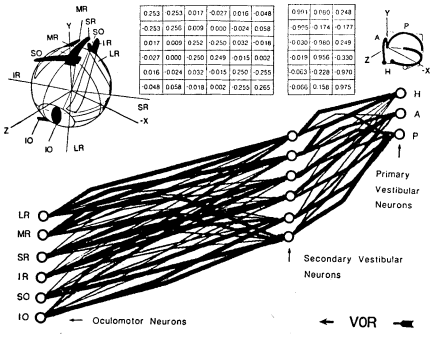
This series of operations bears little resemblance to the classical three neuron direct pathway which simply transmits a reversed head velocity signal to the eyes. However, the direct path can be incorporated in the present model by rearranging the equations to give

$$\boldsymbol{\omega} = -(\mathbf{x}\cdot\mathbf{g}/|\mathbf{g}|^2)\mathbf{R} + \mathbf{T} \times \mathbf{g}/|\mathbf{g}|^2 + (\mathbf{R}\cdot\mathbf{g}/|\mathbf{g}|^2)\mathbf{e} = a\mathbf{R} + \mathbf{b} + \mathbf{c}.$$

In this form, the system can be divided into two parts: a path transmitting **R** and side loop where a, **b** and **c** are computed. The side loop acts on the **R** signal, multiplying it by a and adding **b** and **c**. For a cyclopean eye at the centre of head rotation, with $\mathbf{T} = 0$, a is always -1 and **b** and **c** vanish. Thus, under the classical assumptions, the model collapses to the classical reflex. (Supported by the Medical Research Council of Canada).

304.18 MORPHOLOGY AND A TENSOR NETWORK MODEL OF THE THREE-NEURON VESTIBULO-OCULAR REFLEX ARC IN THE CAT. W. Graf and A. Pellionisz. The Rockefeller University, New York NY 10021, and Dept. Physiol. and Biophysics, New York Univ. Medical Center, New York NY 10016.

The "three-neuron arc" of the vestibulo-ocular reflex connects the semicircular canals of the labyrinth to the eye muscles via primary vestibular afferents, second order vestibular neurons, and oculomotor neurons. The coordinate axes represented by the optimal rotational axes of the semicircular canals and those of the extraocular muscles are available in a quantitative manner. Morphological and physiological data show the principal, accessory and tertiary connections within the reflex arc. A tensor model of the cat vestibulo-ocular reflex arc provides an interpretation of data relating to internal characteristics of CNS networks, resulting in a suitably distributed quantitative model of an overcomplete transformation from three canals to six extraocular muscles:




Neuronal networks in this tensor model accomplish the transformation of a covariant sensory reception vector on primary vestibular afferents into a covariant motor intention vector on second order vestibular neurons, and to a contravariant motor execution vector on oculomotor neurons. The excitatory and inhibitory connections, shown above by the components in two matrices are visualized by line thickness in the corresponding networks. The overall transformation provides a good prediction of the vestibulo-ocular reflex networks as obtained by neuroanatomical and physiological experiments.

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304.19 LIMBIC CORTEX NEURONAL ACTIVITY ASSOCIATED WITH SACCADIC EYE MOVEMENTS IN AWAKE RABBITS AND POSSIBLE UNDERLYING AFFERENTS. R. W. Sikes, B. A. Vogt and H. A. Swadlow . Depts. of Anatomy and Physiology, Boston University School of Medicine, Boston, MA 02118 and Dept. of Psychology, University of Connecticut, Storrs, CN 06268.

Cingulate cortex, a major component of the limbic system, has been associated with complex phenomena such as learning, memory and affective responses to pain. While sensory evoked responses of cingulate neurons have been reported, they are nonspecific and habituate rapidly. Furthermore, cingulate neuronal responses have not been linked to any specific motor response in naive animals. This report describes a class of cingulate cortex neurons with responses which are rigorously and consistantly linked to saccadic eye movements in rabbits. Connections to the oculomotor system which may subserve these responses are also described.

Single unit recordings were made in alert, restrained Dutch belted rabbits. Eye movements were elicited by vestibular and optokinetic stimulation, and eye position was determined by detecting the position of a light emitting diode attached to the eye.

A total of 242 units were isolated in posterior cingulate cortex (area 29), and 11% showed clear responses associated with saccadic eye movements. The majority(75%) responded with a short burst of action potentials 20 to 60 msecs after the onset of each saccade. Half of these units were tested with vestibular stimulation in both light and dark, and the responses were equally vigorous. Many units were directional, most responding preferentially to contralaterally directed saccades. The remaining eye movement sensitive units have longer latency responses which appear to be linked to the offset of each saccade. Lesions marking the location of eye movement sensitive units showed that they were located primarily in area 29d either in the superficial portion of layer II-III or at the border of layers V and VI.

Injections of horseradish peroxidase were made into area 29d to determine possible sources of the eye movement signal. Labeled neurons were located in two areas which have been previously associated with eye movement, cortical area 8 and the lateral thalamic nuclei. Neurons in area 8 were only labeled following rostral area 29d injections, but eye movement sensitive neurons were distributed throughout area 29d. Neurons in the lateral thalamic nuclei, however, overlap the distribution of neurons which have been reported to respond to saccadic eye movements. Thus, the eye movement signal may be thalamic in origin.

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304.20 NEUROANATOMIC IDENTIFICATION OF A RAPHE NUCLEUS IN THE PONS ASSOCIATED WITH OMNIPAUSE NEURONS OF THE OCULOMOTOR SYSTEM IN THE MONKEY. J.A. Büttner-Ennever* and M.Pause* (SPON: H.G. Ross). Brain Research Institute and Neurological Clinic, University of Düsseldorf,FRG.

Omnipause neurons (OPNs) are considered to form a gating-system which controls the generation of saccades. The neurons are known to lie clustered around the midline in the caudal pons. We report here several lines of evidence which show, that OPN-activity is associated with a specific group of raphe neurons which can be considered as a cytoarchitectural entity. According to the classification of Taber et al. ( J. comp. Neurol . 114 :161, 1960) cell groups near the midline in the pons could be divided into: raphe dorsalis lying above and between medial longitudinal fasciculus, raphe pontis in which cells form distinct clusters, raphe magnus lying ventrally and whose cells resemble the adjacent reticular formation. Interposed between these three nuclei lies, each side of the midline, a narrow row of regularly spaced medium-sized neurons, at the level of the abducens rootlets as they pass through the pontine reticular formation. Unlike raphe pontis and raphe magnus, these cells have horizontally oriented dendrites and can be stained in a Golgi-like fashion with cytochrome-oxidase sensitive methods, indicating a high mitochondrial content. We suggest that this group of neurons be called nucleus raphe interpositus (rip). It can also be identified in rat, cat and man. Single OPNs were identified from microelectrode recordings in awake monkeys, and electrolytic lesions were made at these sites. All OPNs had a high tonic discharge rate (100 - 150 spikes/s) which was interrupted 13 + 7 ms before every saccade. The lesions lay within the borders of the newly defined raphe interpositus. In autoradiographic studies of structures involved in saccadic generation afferent projections to the omnipause region were studied. Only raphe interpositus, and not raphe pontis or raphe magnus received afferents from the deeper layers of the superior colliculus, a site known to send monosynaptic inputs to OPNs (Raybourn M.S.,Keller E.L., J.Neurophysiol ., 40 :861,1977); and a specific saccade-related region of the mesencephalic reticular formation (Cohen et al., Exp.Brain Res ., 57 :605,1985) projects heavily to raphe interpositus but not to the surrounding reticular formation. In the light of these results it appears that OPNs are confined to a group of neurons which differ from raphe pontis and raphe dorsalis and raphe magnus with respect to connectivity and cytoarchitecture. Up to now the cell group has been assigned to raphe pontis or raphe magnus, but now deserves a separate name - nucleus raphe interpositus is suggested here. Supported by DFG/SFB 2oo/A3.

304.21 MECHANISMS OF ACCOMMODATION IN THE CHICKEN. D. Troilo* and J. Wallman (SPON: J. Osinshak), Dept. of Biology, City College, City University of New York, New York, NY 10031

It is unclear how similar avian accommodation is to that of mammals. Controversy exists on two points: (1) Does a change in corneal curvature contribute to accommodation? (2) Is the lens actively squeezed by the ciliary processes, rather than simply relaxing into a more curved state as suggested for humans by Helmholtz?

To address these questions, accommodation was elicited in 4-week-old chicks either by electrical stimulation of the Edinger-Westphal nucleus (EW) or by the topical application of 0.4% nicotine sulfate to the cornea. Refractive changes were monitored with a Hartinger refractometer modified for the small eyes of chickens. Changes in the curvatures of the ocular refracting surfaces were measured with keratometry and photography of the Purkinje images. Ultrasonography was used to determine the axial dimensions of the various ocular components. From these data, schematic eyes for both resting and accommodating states were calculated.

We find that a change in corneal curvature is an integral component of accommodation. Data from keratometry and Purkinje image photography indicate that the corneal surface becomes more curved during accommodation. Ray tracing calculations show increases in corneal surface power of 3.71 D for EW stimulation and 6.14 D for topical nicotine treatment. The mean amplitude of accommodation is 9.16 D for EW stimulation and 15.06 D for nicotine application. Although both corneal and total refractive changes are greater with nicotine, the ratios of corneal power change to total refractive change for the two methods are nearly the same (0.40 vs. 0.41) implying that the mechanism by which refractive change is produced is the same for both techniques.

With respect to the mechanism by which lens shape is changed by the ciliary muscle, we find that during accommodation, both the curvature of the anterior lens surface and the thickness of the lens are less than for lenses in vitro. This finding indicates that lens changes during accommodation could have been produced by the release of normal zonular tension although we cannot exclude the possibility that active lenticular squeezing is at work.

Finally, preliminary experiments show markedly reduced accommodative changes in birds older than 1.5 years. This suggests that chickens may become presbyopic. (Supported by NIH EY-02727)

304.22 COMPENSATORY EYE MOVEMENTS PRODUCED BY THE ISOLATED CNS OF THE LARVAL FROG (RANA CATESBEIANA). D.J. Stehouwer. Dept. Psychol. and the Center for Neurobiol. Sci., Univ. Florida, Gainesville, FL 32611.

Undulatory swimming of larval frogs (tadpoles) results from rhythmic bursts of activity that alternate between motoneurons innervating the axial muscles on the left and right sides of the trunk and tail. The resulting undulations propel the animal through the water and produce lateral oscillations of the head. In order to maintain a stable gaze, the eyes must oscillate around the vertical axis to offset movement of the head. It was hypothesized that these conjugate compensatory eye movements are, like the alternating contractions of the axial muscles, generated at least in part by pattern-generating circuits endogenous to the central nervous system (CNS).

This study was conducted to determine whether compensatory eye movements accompany "fictive locomotion" in the absence of vestibulo-ocular and optokinetic reflexes. In the first experiment, "compensatory" eye movements were observed in a radically reduced preparation consisting of a nervous system isolated from all but the head of the tadpole. Rotation of the eyes in a rostral direction accompanied bursts of activity in homolateral motoneurons innervating the axial muscles, and caudal rotation of the eyes was occasioned by bursts of activity in heterolateral spinal motoneurons. The direction and timing of eye rotations were such that they would have compensated for movement of the head, had it occurred.

In the second experiment, electrophysiological recordings from the oculomotor (III), trochlear (IV), and abducens (VI) nerves of completely isolated nervous systems revealed 1:1 coordination between rhythmic bursting of these nerves and that of spinal motoneurons innervating the axial muscles. Bursts in the oculomotor nerve occurred approximately in synchrony with those of the trochlear nerve, whereas bursts of abducens occurred in antiphase with those of the trochlear and oculomotor nerves. Electrical stimulation of the oculomotor nerve resulted in rotation of the eye in an anterior direction about the vertical axis and rotation of the dorsal pole in an anterior direction about the horizontal axis. Stimulation of the trochlear nerve had little effect other than rotation of the dorsal pole of the eye caudally about the horizontal axis. Stimulation of the abducens nerve resulted in retraction of the eye and rotation caudally about the vertical axis.

These results demonstrate that compensatory eye movements can be produced by an isolated CNS without benefit of vestibulo-ocular or optokinetic reflexes, and are part of a larger integrated motor program that also includes the spinal pattern generators for undulatory swimming. (Supported by NS 19720)

304.23 EFFERENTS OF THE NUCLEUS INTERSTITIALIS IN ANURANS. Neil M. Montgomery. University of Massachusetts, Amherst, Massachusetts, 01003.

In his classic study of the amphibian nervous system J. Herrick ('48) described the cell groups surrounding the oculomotor (n.III) and trochlear (n.IV) nuclei as the nucleus interstitialis of the medial longitudinal fasciculus (nInt.); however, little is known about the connectivity of these cell groups in any nonmammal. Potentially these systems could yield valuable information on the nature of sensori-motor integration in the nonmammalian oculomotor system. To this end, the antrograde and retrograde transport of HRP was used to determine the efferent connections of nInt. in Rana pipiens.

HRP was injected into the cell groups surrounding the oculomotor and trochlear nuclei and the antrograde transport of HRP was charted. Antrogradely labelled axon terminals were observed in the (1) tectum, (2) pretectum, (3) cerebellum, (4) vestibular nuclei, (5) spinal cord, (6) contralateral nInt. and (7) across the ventral medulla along the course of the medial longitudinal fasciculus. Each of these regions was then injected with HRP and the distribution of retrogradely labelled cells in nInt. was charted.

As a result of these injections it proved possible to subdivide nInt. based on its differential efferent projections. Further, these subdivisions corresponded to those described by Tuge ('32) in turtle as areas nInt. A, nInt. B, and nInt. C. Tuge divided nInt. based on its cytoarchitecture with area A the most ventral division and area C the most dorsal. In the present study area A nInt. in Rana pipiens was found to project (1) to the spinal cord bilaterally, (2) to the cerebellum, and to the (3) vestibular nuclei. Area B nInt. projects to the (1) ipsilateral pretectal nucleus lentiformis mesencephali, (2) the contralateral nInt. and (3) the ipsilateral spinal cord. Axonal collaterals were also observed in the ipsilateral nIII and nIV. Cells in area C nInt. project to the (1) ipsilateral spinal cord and (2) the deep layers of the optic tectum.

The determination of potential homologies between these structures and those found in mammals will have to await future research and careful comparisons across a wide range of species;however, it appears significant that this region projects to so many sensory nuclei related to ocular reflexes.

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304.24 CENTRIFUGAL FIBERS OF THE OPTIC NERVE CARRY EYE MOVEMENT SIGNALS. J. C. Letelier*, J. Wallman and G. Marin*. Dept. of Biology, City College, City Univ. of N.Y., New York, NY 10031

Like nearly all vertebrate sensory structures, the retina receives centrifugal fibers from the central nervous system. In birds, this projection is especially prominent and arises from the isthmo-optic nucleus (ION), which is known to receive a retinotopic projection from the optic tectum and to project almost exclusively to the retina, where the axons synapse on amacrine cells and displaced retinal ganglion cells. Afferents to the ION from the abducens and trochlear nuclei have also been reported. Previous electrophysiological studies of the ION, using anesthetized animals, have shown responses to visual stimuli, particularly ones moving temporal-to-nasal in the visual field.

We have recorded single units in the ION of alert, young chickens, while recording eye movements using the magnetic field/search coil method. We find that the neurons show, in addition to the expected visual responses, a strong saccade-related response. During saccades, units in the ION nearly cease firing, and immediately following saccades, they give a brief burst of spikes. This suppression of activity during saccades occurs in the dark and therefore cannot be due simply to the smearing of retinal images by the rapid movement of the eyes during saccades.

We suggest that this eye-movement-related centrifugal activity may be analogous to the action of the centrifugal fibers in the lateral line system of fish, in which afferent sensitivity is reduced during self-generated movements. The association of the centrifugal fibers with displaced retinal ganglion cells may, in addition, imply an involvement of the ION with the optokinetic system. (Supported by NIH EY02937.)

## CATECHOLAMINES: RECEPTORS II

305.1 [$^3$H]CLONIDINE BINDS AT MULTIPLE HIGH AFFINITY SITES IN HUMAN PREFRONTAL CORTEX. M. A. Carlson*, A. C. Andorn*, J. E. Piletz*. (SPON: A. Halaris). Depart. of Psychiatry, Case Western Reserve Univ. Schl. of Med. and Cleveland Metropolitan General Hospital, Cleveland, Ohio 44109

[$^3$H]Clonidine binds to particulate fractions of human prefrontal cortex in a process that demonstrates high affinity, saturability, reversibility and selectivity. At 37°C specific binding (determined as the difference between [$^3$H]clonidine binding in the absence and presence of 1 uM clonidine) reaches a maximal value at 10-15 min but a 50% decrease in specific binding occurs between 15 and 60 min at this temperature. [$^3$H]Clonidine binding reaches a steady-state after 20 min at 21°C and the steady-state is maintained for at least 2 hours. The results reported below were obtained at 21°C. Rate dissociation studies were at least biphasic with $t_{1/2}$s of 1.6 +/- a standard deviation of 0.7 and 9.3 +/- 1.1 min. Saturation analyses at 60 min incubation were at least biphasic when plotted according to Scatchard. The approximated apparent $K_D$'s of the extremes were 0.4 +/- 0.1 and 2.4 +/- 0.9 nM with respective approximated $B_{max}$ of 54.0 +/- 20.0 and 126.0 +/- 40.0 fmol/mg protein. Dose-response studies revealed that clonidine was the most potent agonist with an $IC_{50}$ of 5.9 +/- 1.3 nM, followed by (-)-epinephrine with an $IC_{50}$ of 16.6 +/- 9.9 nM followed by (-)-norepinephrine with an $IC_{50}$ 30.7 +/- 4.5 nM. Serotonin had an $IC_{50}$ of 23,500.0 +/- 8,200 nM. Yohimbine was the most potent antagonist with an $IC_{50}$ of 34.0 +/- 10.7 nM and prazosin had an $IC_{50}$ of 4,150.0 +/- 1980.0 nM. In all cases Hill coefficients varied from 0.4 to 0.8. These results indicate that [$^3$H]clonidine binds with high affinity at potentially multiple affinity states or sites in human prefrontal cortex, with a predominant alpha$_2$-adrenergic selectivity.

305.2 DIFFERENTIAL BINDING PROPERTIES OF TWO ALPHA$_2$-ADRENERGIC ANTAGONISTS IN RAT BRAIN. C.L. Boyajian*, S.E. Loughlin and F.M. Leslie (SPON: R.I. Cone). Department of Pharmacology, University of California, Irvine, CA 92717.

The pharmacological characteristics and autoradiographic distribution of two selective alpha$_2$-antagonists, [$^3$H]rauwolscine (RAUW) and [$^3$H]idazoxan (IDAZ), have been examined. Cryostat-cut brain sections (20μm) were incubated with [$^3$H]RAUW or [$^3$H]IDAZ for 2 hr, at 0-4 °C, in Na-K phosphate buffer, pH 7.7. Incubations were performed in the absence and presence of phentolamine (10 μM) to determine specific binding. For autoradiography, radioligand binding sites were visualized by exposure of tissue sections to tritium sensitive film. For radioligand binding assay, tissue sections were scraped from slides into vials to quantify radioactivity by scintillation spectrometry. Scatchard analysis of saturation curves revealed biphasic plots for both radioligands. Concentrations of radioligand which selectively labelled high affinity sites were consistently used in autoradiography and binding assay experiments. The autoradiographic distribution of [$^3$H]RAUW and [$^3$H]IDAZ were distinct and largely non-overlapping. While [$^3$H]IDAZ binding paralled known regions of noradrenergic innervation, [$^3$H]RAUW binding corresponded closely to dopamine terminal fields. Since recent evidence has suggested that rauwolscine possesses antidopaminergic activity (Scatton, B. et al., Eur. J. Pharmacol., 86:427, 1983), we have extensively characterized the pharmacological properties of this binding site. With the exception of idazoxan displacement of [$^3$H]RAUW, dose-response curves for inhibition of radioligand binding were monophasic with Hill slopes of unity. The rank orders of potency for inhibition of [$^3$H]RAUW and [$^3$H]IDAZ binding by endogenous monoamines were similar, i.e., norepinephrine = epinephrine ≫ serotonin = dopamine. Dose-response curves for inhibition of radioligand binding by numerous dopamine antagonists provided further evidence that [$^3$H]RAUW lacked affinity and selectivity for dopamine receptors. The $K_I$ values for inhibition of both [$^3$H]RAUW and [$^3$H]IDAZ binding by dopamine D1 and D2 antagonists were two to three orders of magnitude higher than for inhibition of [$^3$H]spiroperidol binding. Alpha$_2$-adrenergic agonists and antagonists inhibited the binding of both radioligands with high affinity. However, the ratios of their $K_I$ values for inhibition of [$^3$H]RAUW and [$^3$H]IDAZ binding (RAUW/IDAZ) varied widely, ranging from 0.06 to 16. Whereas alpha$_1$-antagonists were more potent displacers of [$^3$H]RAUW than of [$^3$H]IDAZ, alpha$_1$-agonists displayed very low affinity for either site. Furthermore, rauwolscine exhibited low potency for inhibition of [$^3$H]prazosin binding ($K_I$=660nM). These combined data provide anatomical and pharmacological evidence that [$^3$H]rauwolscine binds to a novel binding site within brain which is distinct from that labelled by [$^3$H]IDAZ, yet which shares common alpha$_2$-adrenergic binding properties. (Supported by NIH grants 18843 and 19319.)

305.3 PLATELET $\alpha_2$-ADRENERGIC RECEPTORS: THE EFFECT OF IN VITRO DESIPRAMINE TREATMENT ON $^3$H-CLONIDINE BINDING. E.S. Werstiuk, S.E. Auffarth* and M. Steiner. Depts. of Neurosciences and Psychiatry, McMaster University, Hamilton, Ont., L8N 3Z5, Canada.

Platelet $\alpha_2$-adrenergic receptor binding parameters have been determined in depressed patients. Reports to date are conflicting, in that some groups have found an increased number (Bmax) of $\alpha_2$-receptor sites in patients, while other groups report no difference between patients and controls. These observed changes in platelet $\alpha_2$-receptors may be specific to certain subgroups of depressed patients, or alternately, they may be secondary, and due to drug effects. This study was undertaken to test if in vitro treatment of platelets with the antidepressant, desipramine (DMI) had an effect on the binding parameters of platelet $\alpha_2$-adrenergic receptors.

Washed platelet suspensions were prepared according to the method of Mustard et al., (Brit. J. Haematol., 1972, 22: 193) from freshly obtained blood. Platelet membranes were prepared by mechanical disruption, and the binding assay was performed at 25°C in Tris-HCl$_2$ buffer solution (50 mM) containing $MgCl_2$ (10 mM) at pH 7.5, and $^3$H-clonidine in a concentration range of 0.1 - 64 nM. The reaction was terminated by centrifugation. Specific binding was defined as the difference between total bound radioactivity and the radioactivity not displaced by $6.4 \times 10^{-5}$M clonidine. $K_D$ and Bmax were calculated by the computer program "Ligand" of Munson and Rodbard (Anal. Biochem., 107: 220, 1980). DMI-treated platelets were obtained by incubating intact platelets in Eagle's medium in the presence of DMI (200 - 700 nM) for 1 hr at 37°C, and membranes were prepared as described above.

In 14 young male subjects (28 ± 2 years) we obtained (Mean ± S.E.M.) values of $K_D$: 10.14 ± 1.95 nM and Bmax: 428 ± 53 fmoles/mg protein. In vitro DMI treatment resulted in significantly increased mean values of Bmax: 614 ± 164 f moles/mg protein. No saturable, specific binding of $^3$H-DMI could be detected in the concentration range 2-16 nM tested. $^3$H-DMI treated platelet membranes however, retained 12% of the initially added radioactivity. This membrane-bound $^3$H-DMI is most likely attached to the imipramine binding sites, and/or partitioned into the platelet membrane nonspecifically. $\alpha_2$-receptors of DMI-treated platelet show significantly increased Bmax values suggesting an alteration in the proportion $\alpha_2$H/$\alpha_2$L states. We are currently investigating the relevance of these findings to the in vivo effects of DMI on platelet $\alpha_2$-adrenergic receptors in depressed patients. (Supported by the St. Joseph's Hospital Foundation. ESW holds a career award from the Ontario Mental Health Foundation.)

305.4 CENTRAL ALPHA-2-ADRENOCEPTORS MEDIATE THE NOCICEPTIVE JAW-OPENING REFLEX. A.L. Curtis* and J. Marwah. Dept. of Life Sciences, Indiana State Univ. and Depts. of Pharmacology, Physiology and Neurobiology, Indiana Univ. Sch. Med., Terre Haute, IN 47809.

The jaw-opening reflex (JOR) elicited by tooth pulp stimulation (TPS) has been utilized as an assay for analgesic drugs. However, the involvement and characterization of adrenoceptors in the JOR has not been previously studied. The JOR in lightly anesthetized rats and rabbits induced by intrapulpal (left maxillary) electrical TPS and quantified by the electromyograms (threshold values) recorded from the ipsilateral digastric muscle was used as the experimental pain index. The threshold for the JOR was significantly elevated by clonidine (6.25-100 ug/kg, I.V.) and was inversely correlated with the frequency of stimulation. All doses of clonidine produced an initial transient pressor response followed by a sustained hypotension and bradycardia. The analgesia elicited by clonidine was antagonized by the alpha-2-adrenoceptor antagonist yohimbine (1 mg/kg, I.V.) but not by the alpha-1-adrenoceptor antagonist prazosin (1 mg/kg, I.V.). Polar alpha-2-adrenoceptor agonists, 4-hydroxyclonidine and naphazoline, did not affect nociceptive thresholds at low doses (up to 50 ug/kg, I.V.). At higher doses (200 ug/kg, I.V.) naphazoline, but not 4-hydroxyclonidine, increased the nociceptive threshold. Naloxone had no effect on clonidine-induced analgesia but did antagonize morphine-induced suppression of the JOR. Reserpine pretreatment did not alter the analgesic effects of clonidine. Yohimbine did not antagonize the elevation of nociceptive threshold produced by pentobarbitol. We propose that clonidine modulates JOR analgesia by specifically activating centrally and postsynaptically located alpha-2-adrenoceptors. (Supported in part by American Heart Association Grants 83-757, 85-118 (E.I.); and NIDA grant DA-03519.)

305.5 $\alpha_2$ RECEPTOR CONTROL OVER NOREPINEPHRINE RELEASE IN RAT THALAMUS. C.W. Bradberry* and R.N. Adams. Department of Chemistry, Univ. of Kansas, Lawrence, KS 66045.

The rat locus coeruleus (LC) is composed of noradrenergic cell bodies which project throughout the forebrain and cerebellum. The release of norepinephrine (NE) from these neurons in several brain regions has been shown to be under two site-specific types of regulation mediated by $\alpha_2$-adrenergic receptors. $\alpha_2$ receptors on the cell body mediate an inhibition of the firing rate of LC neurons (Cederbaum, J.M. and Aghajanian, G.K., Eur. J. Pharm., 44:375, 1977). There are also $\alpha_2$ receptors localized at the nerve terminals which mediate a negative feedback regulation of NE release (Langer, S.Z., Br. J. Pharm., 60:481, 1977). This latter form of regulation appears to occur in rat cortex (Wemer, J. et al., J. Pharm. Exp. Ther. 211:445, 1979) and hippocampus (Frankhuyzen, A.L. and Mulder, A.H., Eur. J. Pharm., 81:97, 1982) as shown by in vitro experiments.

Recently, studies have indicated that the presence of autoreceptor regulatory mechanisms in dopaminergic systems depend on the site of projection (Chiodo, L.A. et al., Neurosci., 12, 1, 1984). The present work investigates the presence of "typical" $\alpha_2$ receptor control over NE release in the thalamus, a region not previously examined for these effects.

Two sets of experiments were performed. The first study examined the effects of α-adrenergic agents on relative NE turnover in the thalamus in vivo. Relative NE turnover was measured by analyzing for thalamic whole tissue content of NE following synthesis blockade by FLA-63. Clonidine (0.1 mg/kg i.p.), an $\alpha_2$ agonist, caused a marked reduction in relative NE turnover. Yohimbine (10 mg/kg i.p.), an $\alpha_2$ antagonist, not only reversed the clonidine effect but caused a substantial increase in relative NE turnover compared to control, suggesting a tonic level of $\alpha_2$ inhibition of NE release.

The second set of experiments investigated the effects of the $\alpha_2$ antagonist yohimbine on potassium-stimulated release of endogenous NE from chopped thalamic tissue. A dose-dependent increase in the release of endogenous NE was observed, indicating the presence of localized $\alpha_2$ receptors regulating the release of NE.

305.6 PHORBOL ESTERS AND ALPHA$_1$-ADRENOCEPTORS: DIFFERING EFFECTS ON OUTWARD CURRENTS IN RAT DORSAL RAPHE NEURONS. Jonathan E. Freedman and George K. Aghajanian. Depts. of Psychiatry and Pharmacology, Yale Univ. School of Medicine, New Haven, CT 06508.

Alpha$_1$-adrenoceptors are known to stimulate phosphatidylinositol (PI) turnover, generating the putative second messengers diacylglycerol (DAG) and inositol trisphosphate. We evaluated this model for the excitatory alpha$_1$ response of serotonergic neurons in the rat dorsal raphe, using extra- and intracellular recording in brain slices.

Phorbol esters, agents which mimic DAG by activating protein kinase C, exerted potent effects on raphe cell firing. In extracellular recordings in the presence of the alpha$_1$-agonist phenylephrine (PE, 2 μM), phorbol-12,13-dibutyrate (20-100 nM) and phorbol-12,-13-diacetate (100-500 nM) fully and reversibly inhibited PE-induced cell firing, while the parent alcohol 4-beta-phorbol was inactive at 1 μM.

Intracellular recordings showed that phorbol esters caused hyperpolarization, a drop in input resistance, and a suppression of the afterspike afterhyperpolarization. Single-electrode voltage clamp experiments revealed that phorbol esters (i) induced an outward (probably $K^+$) current, (ii) suppressed the transient $Ca^{2+}$-activated $K^+$ current, $I_{K(Ca)}$, and (iii) suppressed the voltage-dependent $Ca^{2+}$ current, $I_{Ca}$. Conceivably, this could result from tonic opening of $I_{K(Ca)}$ (i and ii), and inactivation of $I_{Ca}$ (iii), known effects of elevated intracellular $Ca^{2+}$. Alternatively, phorbol esters might suppress $I_{K(Ca)}$ and $I_{Ca}$, and induce another, unidentified outward current.

We have previously found that the major excitatory effects of alpha$_1$-receptors are due to suppression of outward currents (resting $K^+$ currents and the A-current), but that these excitatory effects are offset or modulated by prolongation of $I_{K(Ca)}$. Our present results: 1) do not support a role for C-kinase in mediating alpha$_1$ excitatory effects, which were not elicited by phorbol esters, and 2) leave unresolved the question of whether phorbol esters and alpha$_1$-receptors could share an effect on $I_{K(Ca)}$, which, if true, may help explain the paradoxical inhibition of alpha$_1$ responses by phorbol esters in this and other tissues.

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305.7 ANTIDEPRESSANT TREATMENT MODIFIES THE FUNCTIONAL INTERACTION BETWEEN β-ADRENERGIC AND YOHIMBINE-SENSITIVE α-ADRENERGIC RECEPTORS IN RAT BRAIN SLICES. A. Pilc* and S.J. Enna. Depts. Pharmacology and of Neurobiology and Anatomy, University of Texas Medical School, Houston, Texas 77025.

Chronic administration of antidepressants alters the synergistic interaction between α- and β-adrenergic agonists as activators of cAMP production in rat brain slices (A. Pilc & S.J. Enna, Proc. IV World Cong. Biol. Psychiat., in press). Experiments were undertaken to characterize further the pharmacological properties of the brain α-adrenergic component and to examine the manner in which antidepressant treatment alters the relationship between these receptor systems. cAMP production was analysed in rat brain cerebral cortical slices using a prelabeling technique. α-Adrenergic receptors were stimulated with 6-fluoronorepinephrine (6-FNE), while isoproterenol (ISO) was used to activate the β-adrenergic component. The tissue was exposed to both agents simultaneously, or to norepinephrine (NE), for assessing the interaction between the two receptor systems. Although 6-FNE (10 μM) caused a modest (∿ 2-fold) increase in cAMP production, ISO (1.0 μM) elevated second messenger levels over 6-fold and the response to the drug combination, or to NE, was a 14-fold enhancement, indicating a synergistic interaction between the α- and β-receptor components. This interaction was only partially (< 30%) inhibited by prazosin, an $\alpha_1$-adrenergic receptor antagonist. In contrast, the $\alpha_2$-antagonist yohimbine completely eliminated the potentiated response, reducing NE-stimulated cAMP production to a level found with ISO alone. The concentration of yohimbine necessary to inhibit 50% of the α-component associated with the NE response ($IC_{50}$) was 1 μM. Chronic (2 wks) administration of 10 mg/kg imipramine, mianserin, or citalopram reduced the synergistic interaction between 6-FNE and ISO. Moreover, drug treatment significantly enhanced the potency of yohimbine to block the α-adrenergic component of the NE response ($IC_{50}$ = 0.5 μM). These data suggest that β-adrenergic receptor-coupled cAMP production in brain slices is potentiated by $\alpha_2$ - adrenergic receptor sites. Also, it appears that antidepressant administration modifies the synergistic interaction between the α- and β-adrenergic receptors, perhaps by altering the receptivity of the yohimbine-sensitive component.
(Supported in part by UPHS grants MH-35945 and MH-00501).

305.8 **MORPHOLOGICAL CHANGE IN RAT ASTROCYTES INDUCED BY A PHORBOL ESTER IS NOT ASSOCIATED WITH CHANGE IN THE SENSITIVITY OF THE β-NORADRENERGIC RECEPTOR-ACTIVATED ADENYLATE CYCLASE SYSTEM.** **P. Mobley. Dept. of Pharmacology, University of Texas Health Science Center, San Antonio, Tx. 78284.**

**A well known feature of astrocytes maintained in culture is their transformation from flat, polygonal cells lacking processes to stellate, process-bearing cells when exposed to analogs of cyclic AMP or agents which increase the intracellular availability of cyclic AMP. These agents can also decrease the sensitivity of the β-noradrenergic-receptor-activated adenylate cyclase system in astrocytes. The results presented here suggest that these two events are independent of each other.**

**Astrocytes, prepared from the neocortex of newborn Sprague-Dawley rats, were grown and maintained in Dulbecco's Modified Eagle's Medium (DMEM) plus serum (10% fetal bovine, 5% calf) at 37°C in an atmosphere of 5% $CO_2$/95% air. At confluency these cells have a flat polygonal shape and lack processes. After exposure to 300μM isobutylmethylxanthine (IBMX) for 2 hrs, a large proportion of the astrocytes were transformed to process-bearing cells. A similar morphological change was observed in cells exposed to 400nM phorbol 12-myristate 13-acetate (PMA) for 2 hrs. The vehicle for PMA, dimethyl sulfoxide (0.05% final concentration), had no effect on morphology.**

**The effect of IBMX or PMA exposure on the isoproterenol-induced accumulation of cyclic AMP in astrocytes was also determined. In cells treated with 300μM IBMX, washed, and then exposed to 1μM isoproterenol for 10 min, the cyclic AMP response was approximately 50% less than in untreated cells exposed to isoproterenol. Treatment with IBMX did not appear to alter basal levels of cyclic AMP. In contrast, in cells treated with 400 nM PMA, washed and then exposed to 1μM isoproterenol for 10 min, no change in the isoproterenol induced accumulation of cyclic AMP was observed compared to vehicle control. (PMA, 2123 ± 149 pmoles cyclic AMP/mg protein; vehicle, 2071 ± 151 pmoles cyclic AMP/mg protein). Treatment with PMA did not appear to alter basal levels of cyclic AMP.**

**These studies suggest that the decrease in isoproterenol-induced cyclic AMP accumulation in cells treated with IBMX is not a consequence of morphological change. In support of this, a 2 hr treatment of astrocytes with isoproterenol causes only slight morphological change but decreases the cyclic AMP response elicited by re-exposure to isoproterenol by 70-90%. Since phorbol esters are activators of protein kinase C, this data also suggests that similar morphological changes can be induced in astrocytes by both the protein kinase C system and the cyclic AMP-dependent protein kinase system.**

305.9 EFFECT OF SUBACUTE CO-ADMINISTRATION OF DESIPRAMINE WITH NE OR 5-HT RELEASING AGENTS ON β-ADRENERGIC AND 5-$HT_2$ RECEPTORS IN RAT BRAIN WITH OR WITHOUT NE NEURON LESION. S.Matsubara*, M.Mikuni, T.Omori*, T.Kazawa* and I.Yamashita* (SPON:J.Tanji) Dept. of Psychiatry, Hokkaido Univ. Schl. of Med.,Sapporo, Hookaido 060, Japan

We investigated the effects of subacute (3 days) combined administration of desipramine(DMI) with various agents which were reported to promote the release of NE (yohimbine(YOH),(+)-mianserin(MIA)) or 5-HT(methiothepine(MET),(+)-MIA,(-)-MIA), on β-adrenergic and 5-$HT_2$ receptors in rat cerebral cortex. These agents were administered to naive or DSP-4 pretreated male Wistar rats. DSP-4, which selectively decreased the concentration of NE in parietal cortex, was kindly gifted form Dr.S.O.Ogren.

Treatment with MET, (+)-MIA or (-)-MIA alone decreased the numbers of 5-$HT_2$ receptor labelled by 3H-spiperone. These changes were more significant when co-administered with DMI. DMI or YOH alone did not change the numbers of 5-$HT_2$ receptor, whereas the combined administration thereof decreased them. DSP-4 pretreatment did not prevent the effects of (+)-MIA and YOH mentioned above. None of these agents changed the numbers of β-adrenergic receptor labelled by 3H-DHA when administered alone, but the combined administration of DMI with (+)-MIA, (-)-MIA or YOH but not with MET decreased the receptor numbers. These changes were abolished by DSP-4 pretreatment which increased β-adrenergic receptor numbers.

(+)-MIA was more potent to down-regulate 5-$HT_2$ receptor than (-)-MIA, although both were reported to be equipotent in 5-HT releasing effect. We previously reported that 5,7-DHT pretreatment did not prevent the down regulation of 5-$HT_2$ receptors induced by DMI. In addition, YOH promoted the DMI-induced down regulation and this effect was not prevented by the destruction of NE neurons. These results suggest the possibility that 5-$HT_2$ receptor numbers are regulated by postsynaptic α-2 adrenoceptor-mediated mechanisms and are not regulated by the availability of the transmitter, 5-HT. Our results concerning β-adrenergic receptors confirm the crucial role of NE in the regulation of the receptor numbers, and also suggest the possibility of postsynaptic α-2 adrenoceptor-mediated mechanisms, since (+)- and (-)-MIA equally promoted the DMI-induced down regulation of β-adrenergic receptors, although (+)-MIA but not (-)-MIA was reported to be active in NE release.

305.10 ALTERED BETA ADRENERGIC RECEPTOR FUNCTION IN A MONOCYTE-LIKE CELL LINE. D. Hill*, T.Wallace*, J.Van Boxel*, R.Schneider*, P.J. Mills*, and R.K.Wallace. Depts. of Neuroscience and Physiological Sciences, Maharishi International University, Fairfield, Iowa, 52556.

The beta adrenergic receptor (BAR) is being investivestigated in our laboratory as part of an ongoing program to assess the relationship between the nervous and immune systems in specific mental states. There are well established associations between a number of disease states and altered catecholamine (CA) levels, physiological responsiveness, and BAR number and/or affinity. For example, subsensitivity to beta adrenergic agonists in asthma and low lymphocyte BAR affinity in hypertension and aging. More recently, researchers are focusing on the connections between the immune and nervous systems as revealed by the weakened immune response in the lymphocytes from depressed and bereaved patients.

As an initial phase of our investigation, BAR is being studied in a series of cell lines by radioligand binding techniques utilizing (-)- Iodocyanopindolol, [125I] (CYP:NEN). In a monocyte-like cell line (u-937 histiocytic lymphoma, human: American Type Culture Collection) the addition of 5'- guanylylimidodiphosphate Gpp(NH)p failed to affect the BAR affinity, thus indicating a homogeneous low affinity BAR population. However, in a control study using freshly isolated peripheral blood lymphocytes, addition of Gpp(NH)p did right shift the competition curve, thus transforming all the BAR into the low affinity state.

The lymphocyte is a readily available model for determining the relationship between the nervous system, and its effect via CA activation of the BAR, and the immune system, which has lymphocytes as one of its primary effector mechanisms.

This model will be employed to examine the influence of mental stress on lymphocyte BAR and immune function, and its modification through the stress management technique of Transcendental Meditation.

305.11 COMPARISON OF BETA-1 AND BETA-2 ADRENERGIC RECEPTORS IN A SINGLE CULTURED CELL SYSTEM. Mark D. Dibner and Mary A. Bailey*. Neurobiology Group. Central Research and Development Department, E.I. du Pont de Nemours and Co., Experimental Station, Wilmington, DE 19898.

The subtypes of beta-adrenergic receptors have been characterized by comparison in different cells or tissues. This study examines receptors within the same system. The rat C6 glioma cell has both β1 and β2 adrenergic receptors, as demonstrated by displacement of [$^{125}$I]-iodocyanopindolol (ICYP) binding by specific β1 and β2 adrenergic antagonists in intact cells. There are 45% β1 receptors and 55% β2 receptors on these cells. By analysis of ICYP binding saturation and displacement as well as stimulation of cyclic AMP, the β1 and β2 receptors were further characterized. The antagonists ICI-89406 and ICI-118551 are highly selective for β1 and β2 receptors, respectively. In our hands they have no agonist activity (i.e., ability to stimulate cyclic AMP formation) at up to 10 μM. Displacement of ICYP binding by either drug yielded a biphasic curve with the IC50 for high affinity in the low nM range, the second IC50 in the μM range and a small flat region at 100 nM. Thus, β1 or β2 receptors were studied in the presence of 100 nM of the β2 or β1 antagonist. Isoproterenol has a higher affinity for displacing ICYP binding at β2 compared to β1 receptors, but it is more potent in stimulating cyclic AMP accumulation (in whole cells, measured by RIA) at β1 receptors than at β2. Dopamine appears to have a higher affinity for β2 receptors, but it is also more potent at stimulating cyclic AMP at the same receptor, compared to the β1 receptor. We recently described the ability of dopamine to desensitize the beta-adrenergic system in C6 glioma cells. When cells are exposed to 10 μM dopamine for 24 hours, there is a 60% loss of ICYP binding, as determined by Scatchard analysis. Both β1- and β2-adrenergic receptors appear to be affected; with more β2 lost than β1. After exposure to dopamine, isoproterenol-stimulated cyclic AMP accumulation is decreased more at β2 receptors than at β1 receptors. These studies demonstrate specific qualitative and functional differences in beta-adrenergic receptor subtypes in the same cell system. (Beta antagonists were a gift from ICI, Ltd.)

305.12 6-HYDROXYDOPAMINE LESIONS INCREASE NOREPINEPHRINE-INDUCED INOSITOL PHOSPHOLIPID HYDROLYSIS IN THE RAT STRIATUM. Marian S. Kafka, Rodrigo Labarca*, Aaron Janowsky*, Julie A. Blendy*, and Steven M. Paul*. Clinical Neuroscience Branch, NIMH, Bethesda, MD20205

The agonist-induced hydrolysis of inositol phospholipids (PI) has been characterized in the rat striatum by measuring the accumulation of [$^3$H]inositol phosphates (IP) in striatal slices and a particulate preparation (1), pre-labelled in vitro with [$^3$H]myo-inositol. Both carbachol (5-6000 μM) and norepinephrine (NE; 0.5 - 100 μM) stimulated PI hydrolysis. Carbachol-induced [$^3$H]IP accumulation was antagonized by atropine and scopolamine, and NE-induced [$^3$H]IP accumulation was antagonized by prazosin and WB4101, in a concentration-dependent manner. The response to carbachol appears to be mediated by muscarinic cholinergic receptors as the nicotinic agonist, citisin, failed to increase [$^3$H]IP accumulation, and the nicotinic antagonist, mecamylamine, failed to inhibit the effects of carbachol. The response to NE appears to be mediated by $\alpha_1$-adrenergic receptors as l-isoproterenol and clonidine failed to stimulate [$^3$H]IP accumulation, and yohimbine and dl-propranolol did not inhibit the effects of NE. Similar pharmacological relationships were observed using a particulate preparation.

Prior treatment with 6-hydroxydopamine (6OHDA; 2 X 200 μg icv., 4 days apart) increased the maximum accumulation of [$^3$H]IP induced by NE without changing the $EC_{50}$ of the NE-induced [$^3$H]IP accumulation. Carbachol-induced [$^3$H]IP accumulation was not altered by the 6OHDA lesion. Lesions produced by a knife-cut of the medial forebrain bundle (1P, 1.5L from bregma) produced effects similar to 6OHDA lesions. As there appear to be few or no noradrenergic nerve endings in the striatum (2), the effects of dopamine on [$^3$H]IP accumulation were examined. Dopamine (1-100 μM) failed to increase consistently [$^3$H]IP accumulation.

Because lesions of the dopaminergic afferents to the striatum increased $\alpha_1$-receptor-mediated stimulation of PI hydrolysis, it is possible that those afferents exert an inhibitory influence on the striatal PI system.

(1) Hollingsworth et al., J. of Neuroscience, In Press
(2) Versteeg et al., Brain Res. 113: 563-574 (1976)

305.13 **QUANTITATIVE AUTORADIOGRAPHY OF RAT CORTICAL $\beta_1$- AND $\beta_2$-ADRENERGIC RECEPTORS AFTER 6-OHDA TREATMENT.** E.W. Johnson*, T.C. Rainbow, and B.B. Wolfe (SPON: G.B. Koelle). Dept. of Pharmacology, Univ. of Penn. Sch. of Med., Phila. PA 19104.

Intraventricular administration of the neurotoxin 6-hydroxydopamine (6-OHDA) to adult rats results in a reduction of forebrain norepinephrine (NE) levels as well as a 30% increase in the density of $\beta_1$ receptors in rat cerebral cortex. Using quantitative autoradiography, we have localized and quantified discrete regional variations in the density of β-adrenergic receptor subtypes in the rat brain after treatment with 6-OHDA. Due primarily to the increase in anatomical resolution afforded by quantitative autoradiography, we have not only been able to confirm the increase in $\beta_1$ receptors seen previously, but have also been able to demonstrate a significant increase in the density of $\beta_2$ receptors in the rat cortex and in other identifiable regions of the rat brain.

Adult rats were injected intracerebroventricularly on two successive days with 10 μl of 0.1% ascorbic acid in normal saline with or without 200 μg of 6-OHDA. The animals were decapitated one week after the initial injection and the brains were used either for determination of NE levels by HPLC with electrochemical detection or they were freeze-mounted onto cryostat chucks, cut into 32-μm-thick sections and thaw-mounted onto subbed glass slides. Sections from a number of anatomical levels were incubated with ($^3$H)-DMI, a selective label for NE uptake sites. Other sections from each level were incubated with ($^{125}$I)-iodopindolol ($^{125}$I-IPIN), a nonselective β-receptor antagonist, ($^{125}$I)-IPIN plus ICI 89,406, a potent and highly selective $\beta_1$ antagonist, ($^{125}$I)-IPIN plus ICI 118,551, a selective $\beta_2$ antagonist or ($^{125}$I)-IPIN plus isoproterenol. This procedure yielded an estimate of the relative numbers of β-adrenergic receptors, $\beta_2$ receptors, $\beta_1$ receptors or nonspecific sites. After labeling, the sections were apposed to LKB ultrofilm to generate autoradiograms. The autoradiograms were subjected to computerized quantitative densitometry, a process that allows conversion of optical density into molar quantity of ligand bound. HPLC analysis of samples from control and 6-OHDA-treated brains indicated that treatment with 6-OHDA resulted in depletion of 85% of the NE in the cerebral hemispheres. A general decrease in the binding of ($^3$H)-DMI was seen in brain sections obtained from 6-OHDA-treated animals, indicating a widespread loss of neurons containing the uptake site for NE.

As seen in previous studies, the density of $\beta_1$ receptors in the cerebral cortex of 6-OHDA-treated animals was increased about 30%. This increase occurred more in somatomotor cortex than in frontal or occipital cortex. In contrast to previous reports, the density of $\beta_2$ receptors was also increased about 40% over control values. This change occurred mainly in frontal cortex. There was also a greater than 200% increase in the density of $\beta_2$ receptors in the caudal portion of the lateral posterior nucleus of the thalamus (LPT). The density of $\beta_1$ receptors did not change in the LPT. (Supported by NIH Grant NS-19597 and Klingenstein Fellowship.)

305.14 BETA-ADRENERGIC RECEPTOR DENSITY AND N PROTEIN COUPLING IN THE CEREBELLUM OF THE RAT AFTER REPEATED TREATMENT WITH CLENBUTEROL. G.A. Ordway*, J.M. O'Donnell and A. Frazer, (SPON: P. Whybrow) Depts. of Psychiatry and Pharmacology, Univ. of Pennsylvania and VAMC, Philadelphia, PA 19104.

Recently, we found that repeated treatment of rats with the centrally-active beta-adrenergic agonist clenbuterol (CLEN) reduced the ability of isoproterenol (ISO) to elevate the concentration of adenosine 3',5'-monophosphate (cyclic AMP) in cerebral cortical slices. This reduced beta-adrenergic responsiveness in the cortex was not accompanied by a reduction in the density of beta-adrenergic receptors but rather was due to an uncoupling of these receptors from the N protein (J. Pharmacol. Exp. Therap., in press). As CLEN has been reported to have beta$_2$-selectivity, it was of interest to compare its effects in the cortex (a brain area with predominantly beta$_1$ receptors) with those found in a brain area containing mostly beta$_2$ receptors, e.g., the cerebellum. Male rats were injected with CLEN (1-10mg/kg, i.p.) or 0.9% NaCl once daily for periods of one to 16 days. Eighteen hours after the final drug administration, the rats were killed by decapitation and homogenates of cerebellum prepared as described previously (J. Pharmacol. Exp. Therap. 228:640, 1984). Saturation experiments were done using the beta-antagonist $^{125}$I-iodopindolol (IPIN) as the ligand to determine the density of beta-adrenergic receptors and their affinity for IPIN. The "coupling" of these receptors to the N protein was inferred from analysis of the competition curves produced by isoproterenol of the binding of IPIN in the absence and presence of GTP.

In contrast to results measured in the cortex, treatment of rats with CLEN caused a reduction in the density of beta-receptors in the cerebellum; this was both dose-and time-dependent. Eighteen hours after a single injection of clenbuterol (10 mg/kg), beta-receptor density was reduced by 50%; after eight days of administration, the reduction in density was about 80%. CLEN treatment did not change the affinity of the beta-receptors in the cerebellum for IPIN, but did uncouple the beta-receptors in the cerebellum from the N protein. The ability of CLEN to reduce the density of beta-receptors in the cerebellum but not in the cortex may be related to its ability to produce greater beta-adrenergic effects in tissues with predominantly beta$_2$ rather than beta$_1$ receptors. (Supported by Research Funds from the VA and USPHS Grants MH29094 and MH14654).

**305.15** RAPID DOWN-REGULATION OF BETA-ADRENERGIC RECEPTORS BY COMBINING ANTIDEPRESSANT DRUGS WITH FORCED SWIM: A MODEL OF ANTIDEPRESSANT-INDUCED NEURAL ADAPTATION. G.E. Duncan*, I.A. Paul*, R.A. Mueller, P.P. Rowell and G.R. Breese. Biological Sciences Research Center, UNC School of Medicine, Univ. of North Carolina at Chapel Hill, Chapel Hill, NC 27514.

The hypothesis that behavioral responses to antidepressant drugs in the forced swim test are related to a rapid neural adaptation produced by the combination of drug treatment and swim stress was explored. As a measure of adaptation, brain beta-adrenergic receptors were assayed using $^3$H-dihydroalprenolol ($^3$H-DHA) binding to brain membranes from rats that were processed in the forced swim test. The combination of swim stress and imipramine treatment antagonized immobility induced by forced swimming and resulted in a reduction in $^3$H-DHA binding to membranes from forebrain preparations which did not include the corpus striatum. Administration of antidepressant drugs from other chemical classes, including pargyline, iprindole, and nomifensine, also reduced immobility induced by the forced swim and produced a reduction in $^3$H-DHA binding to forebrain membranes. In homogenates of the corpus striatum, $^3$H-DHA binding was not altered by swim stress combined with antidepressant drug treatment. Chlordiazepoxide was without an effect on immobility or beta receptor binding when combined with forced swim. Even though atropine and amphetamine exhibited a positive activity in the forced swim test, they did not reduce $^3$H-DHA binding. Therefore, by combining behavioral and neurochemical analysis of animals processed in the forced swim test, it may be possible to differentiate, with greater confidence, potential antidepressant drugs from "false positives". The present studies support the hypothesis that antidepressant drug action in the forced swim test involves a rapid neural adaptation, as reflected by the down-regulation of beta-adrenergic receptors. Thus, this behavioral paradigm may serve as a model of adaptive mechanisms induced by antidepressant drugs.

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**305.16** MEASUREMENT OF IN VIVO BETA ADRENOCEPTOR TURNOVER IN THE RAT STRIATUM. B.L. Largent, A.L. Gundlach, J. Pitha* and S.H. Snyder. The Johns Hopkins University School of Medicine, Department of Neuroscience, *National Institute on Aging, GRC-Francis Scott Key Medical Center., Baltimore, MD.

Recently, an irreversible beta adrenoceptor (BAR) antagonist, bromoacetylalprenololmenthane (BAAM), has been synthesized (Pitha, J. et al., Life Sci. 27:1791, 1980) and characterized against peripheral BARs (Baker, S. and J. Pitha, J. Pharm. Exp. Ther. 220:247, 1982). We have characterized the interaction of BAAM with CNS BARs. Additionally, BAAM was utilized to measure the in vivo turnover rate for BARs in the rat striatum.

BAAM inhibited 125-I-Cyanopindolol (ICYP) binding to BARs in rat cortex, cerebellum, and striatum ($IC_{50}$ of ~ 1-3 nM) with no apparent selectivity for β-1, or β-2 adrenoceptor subtypes. BAAM inhibition of 125-ICYP binding demonstrated two components - a competitive portion and an irreversible portion. The extent of alkylation of BARs by BAAM was time and concentration dependent.

BAR metabolism rates can be measured by irreversibly blocking a portion of a population of BARs and then monitoring the recovery of that population toward steady state levels. Assuming a monoexponential process for the repopulation kinetics, a plot of the data ([Rss] = steady state receptor level and [Rt] = receptor level at time t) as ln ([Rss]/[Rss]-[Rt]) vs time will provide an indication of the t 1/2 for recovery, the synthesis rate, and the degradation rate for the receptors. Since BAAM does not appreciably cross the blood-brain barrier, our studies measuring CNS BAR turnover required direct injection of BAAM into the CNS to alkylate BARs.

Stereotaxic microinjection of BAAM (20 µg/0.2 µl EtOH) into the rat striatum decreased the BAR levels to 25-30°/° of control values, until approximately 30 days after which a recovery period began. After 135 days, an apparent recovery plateau was reached at approximately 60°/° of control levels. At this point it is not clear if receptor level recovery would continue - at a much slower pace - eventually reaching control levels or remain at that degree of recovery. Assuming the control receptor level (~ 135 fmols/mg protein) equals Rss, plotting the data gives the following metabolism parameters: synthesis rate = 1.42 fmols/mg protein-day, degradation rate = 0.0104 $day^{-1}$, and a t 1/2 = 66 days. However, using the apparent plateau recovery level (85 fmols/mg protein) as Rss provides the metabolism parameters: synthesis rate = 1.98 fmols/mg protein-day, degradation rate = 0.0233 $day^{-1}$, and a t 1/2 = 30 days. The comparison of metabolism rates in control receptor states to modulated receptor states, such as following chronic drug treatments, should provide new insight into the regulation of receptors.

**305.17** CHARACTERIZATION OF THE β-ADRENORECEPTOR ON RAT BROWN ADIPOCYTES. B.E. Levin and A.C. Sullivan*. Neurology Service, Veterans Administration, E. Orange, NJ 07019, Dept. of Neurosciences, N.J. Medical School, Newark, NJ 07103, Dept. of Pharmacology, Hoffmann-LaRoche, Nutley, NJ 07110.

Brown adipose tissue (BAT) is a major source of sympathetically controlled thermogenesis in the rat as stimulated primarily by β-adrenoreceptors. The β-subtype has been in doubt because of the difficulty of carrying out detailed binding studies with previously available ligands, all of which were lipophylic, yielding high levels of non-specific binding. We used ($^3$H)CGP 12177 (4-3-t-butylamino - 2-hydroxypropoxy)-{5,7-$^3$H}benzimidazol-2-one), a hydrophilic ligand, to characterize the β-receptor in BAT. Binding was carried out in 5 mM Hepes buffer containing 1 mM $MgSO_4$, pH 8.0 at 37°C for 20 min. Specific binding in the presence of 30 µM isoproterenol was 60-80% of total. Binding to collagenase-separated whole cells gave: $K_d$ = 323 ± 28pM, $B_{max}$ = 26.7 ± 1.4 x $10^{-5}$fmol/cell; binding to partially purified membranes from isolated adipocytes gave: $K_d$ = 499 ± 53 pM, $B_{max}$ = 14.4 ± 0.86 fmol/mg protein. Results of competitive binding studies in purified BAT membranes with various $\beta_1$- and $\beta_2$-specific ligands (in the presence of 1 nM ($^3$H)CGP and 100 µM GTP) showed there to be at least 2 subtypes (about 80% $\beta_1$-, 20% $\beta_2$-):

| | $\beta_1$- | | $\beta_2$- | |
|---|---|---|---|---|
| | $K_d$ | % | $K_d$ | % |
| $\beta_1$-specific | | | | |
| ICI 89409 | 4.40 ± 0.37nM | 83.8 ± 2.6 | 0.36 ± 0.20µM | 16.2 ± 3.0 |
| Metoprolol | 23.4 ± 5.2 nM | 81.2 ± 8.1 | 0.95 ± 0.11µM | 18.9 ± 7.2 |
| $\beta_2$-specific | | | | |
| ICI 118551 | 1.29 ± 0.08µM | 78.9 ± 11.8 | 2.63 ± 0.47nM | 21.1 ± 7.9 |
| Zinterol | 0.68 ± 0.07µM | 82.9 ± 2.0 | 4.87 ± 2.71nM | 17.1 ± 2.5 |
| Other | | | | |
| NE | 1.13 ± 0.03µM | 83.2 ± 2.0 | 395 ± 26 µM | 16.8 ± 2.1 |
| LY 22-8899 | 2.01 ± 0.41µM | 89.1 ± 4.8 | 8.83 ± 1.24nM | 10.9 ± 4.2 |

The latter compound (LY 22-8899) is a thermogenic and lipolytic agent which has its highest affinity for the $\beta_2$-receptor. Nevertheless, there was no evidence for a 3rd type of β-receptor as suggested by the in vitro physiological studies of Arch et al. (Nature 309: 143, 1984); the $\beta_1$-receptor appeared to be the predominant subtype by these competitive binding studies.

**305.18** CHARACTERIZATION OF DOPAMINE RECEPTORS FUNCTIONALLY LINKED WITH CALCIUM CHANNELS. M. Memo, L. Castelletti*, A. Valerio*, C. Missale, M.O. Carruba*, P.F. Spano. Inst. of Pharmacol. Exp. Ther., Sch. of Med., Univ. of Brescia and Dept. of Pharmacol., Chemiother. and Med. Toxicol., Univ. of Milano, Italy.

Dopamine (DA) is a well known prolactin inhibitor factor which can be released in the tubero-infundibolar system and then inter acts with specific receptors in the pituitary cells. It has been suggested that DA decreases prolactin release by modulating in an inhibitory way basal or stimulated adenylate cyclase activity. We now report that DA can prevent the release of prolactin induced by neurotensin by interacting with DA $D_2$ receptors which appear to be completely independent of adenylate cyclase moiety, but are functionally coupled to calcium channels.

Exposure of pituitary cells in continuously perfused columns for 10 min to 50 nM DA prevented the neurotensin-induced increase of prolactin release. This effect of dopamine was dose-dependent with a half maximal inhibitory effect at 5 nM. Like DA, various DAergic agonists such as bromocriptine, lisuride and apomorphine inhibit neurotensin-induced prolactin release. In the same experimental conditions, 50 nM DA completely inhibits neurotensin-induced increase of calcium influx into the cells. The $IC_{50}$ of DA for this effect was 8 nM. The inhibitory action of dopamine was mimicked by bromocriptine, lisuride and apomorphine, with $IC_{50}$ values of 2.5 nM, 7.0 nM and 50 nM, respectively. The inhibitory action of DA was stereospecifically reversed by sulpiride. In the range of concentrations that are effective in inhibiting either the calcium influx and prolactin release,both induced by neurotensin, DA does not alter the cyclic AMP generating system. 50 nM DA does not affect adenylate cyclase activity in rat pituitary gland homogenates and does not modify intracellular concentrations of cyclic AMP in dispersed cells. Moreover, the inhibitory effects of DA on neurotensin-induced both calcium entry and prolactin release, persisted in pertussis toxin-treated cells. The effect of DA appears to be specifically linked to receptor-operated, rather than voltage-operated, calcium channels. DA (50 nM) did not alter the entry of calcium induced by high potassium concentrations or veratridine.

Our results indicate that DA $D_2$ receptors in anterior pituitary are, at least in part, functionally coupled with calcium channels.

306.1 QUANTITATIVE DISTRIBUTIONS OF ASPARTATE AMINOTRANSFERASE AND GLUTAMINASE ACTIVITIES IN THE COCHLEA. G.J. Wiet*, D.A. Godfrey, C.D. Ross and J.D. Dunn. (SPON: D.F. Peterson), Depts. Physiol. and Anat., Oral Roberts Univ., Tulsa, OK 74171.

Aspartate aminotransferase (AAT) and phosphate-dependent glutaminase (PDG), prominent enzymes of aspartate and glutamate metabolism, have been suggested to be important in neurons that may release these amino acids as transmitters. To evaluate possible roles of AAT and PDG in hearing, quantitative microchemical techniques have been applied to study their distributions in the cochlea. Also assayed was choline acetyltransferase (ChAT), the enzyme synthesizing acetylcholine in cholinergic neurons, and a marker for the auditory feedback pathway to the cochlea from the brain stem: the olivocochlear bundle (OCB). Young rats (300-400 g) and guinea pigs (160-200 g) were decapitated, and the temporal bones quickly removed, frozen in Freon chilled with liquid nitrogen, and freeze-dried for one week. Cochleas were dissected by a three-dimensional approach. Samples of inner and outer hair cell regions and outer supporting cell region of the organ of Corti, Reissner's membrane, tectorial membrane, and stria vascularis were removed, weighed (0.02-0.5 μg) and then assayed for AAT, PDG, or ChAT activity. AAT activity was about the same in both synaptic (hair cell) and non-synaptic (supporting cell) zones within the organ of Corti, was almost twice as high as this in the stria vascularis, and was much lower in Reissner's and tectorial membranes. PDG, on the other hand, had its highest activity in synaptic zones, about half that activity in organ of Corti non-synaptic zones, and almost no activity in Reissner's and tectorial membranes and stria vascularis. ChAT activity was highest in synaptic zones, with activities close to 100 times those of the other, non-neural, structures. Seven days after unilateral transection of the OCB in the brain stem of three rats, no significant difference was found between lesion- and control-side AAT and PDG activity, even though the activity of ChAT in the organ of Corti fell to zero. Both the distribution of AAT activity and the lesion results would seem to implicate AAT in an energy metabolism role moreso than a neurotransmitter role in the cochlea. The relatively high activity in the stria vascularis correlates with relatively high activities of other oxidative metabolic enzymes, such as malate dehydrogenase. The distribution of PDG would be consistent with a possible role in neurotransmission; however, the lesion data suggest that it is not associated with the olivocochlear bundle.

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306.2 LOCALIZATION OF GLYCINERGIC SYNAPSES IN THE COCHLEAR NUCLEUS AND SUPERIOR OLIVARY COMPLEX WITH MONOCLONAL ANTIBODIES SPECIFIC FOR THE GLYCINE RECEPTOR. R. J. Wenthold, H. Betz, K. A. Reeks*, M. H. Parakkal* and R. A. Altschuler. Lab. of Neuro-otolaryngology, NINCDS, NIH, Bethesda, MD 20205 and Univ. of Heidelberg, Federal Republic of Germany.

Glycine is believed to be a major inhibitory neurotransmitter with highest levels found in the spinal cord and brainstem. Previously, synapses using glycine as a transmitter could not be readily localized because of the lack of a specific immunocytochemical marker. However, the postsynaptic glycine receptor has been purified and monoclonal antibodies produced against it, providing a specific marker for glycinergic synapses (Pfeiffer et al., PNAS 81, 7224). Biochemical and pharmacological evidence suggests that glycine is a neurotransmitter in the auditory system. To determine the precise localization of glycinergic synapses, the immunocytochemical distribution of the glycine receptor was determined in the cochlear nucleus and superior olivary complex.

Rats and guinea pigs were used in the study. For light level localization animals were perfused with paraformaldehyde, 0.1-4%, and for electron microscopic localization, animals were perfused with 4% paraformaldehyde and 0.1% glutaraldehyde. Labeling was determined with both immunofluorescence and immunoperoxidase techniques. At the light microscopic level, two different monoclonal antibodies (GlyR 5a and 7a) gave the same immunocytochemical labeling. Labeling was seen throughout the cochlear nucleus, appearing as puncta often present around cell bodies. Electron microscopic studies in the AVCN were done with GlyR 7a. These show that labeling is restricted to the postsynaptic region of both axo-dendritic and axo-somatic synapses. Several different terminal populations have been described in the AVCN based on vesicle shape and size and terminal morphology. Preliminary analyses suggest that glycine receptors are apposed to terminals containing flattened vesicles. Immunoreactive label was present on cell bodies and processes in nuclei of the superior olivary complex including the medial and lateral superior olivary nuclei and medial and ventral nuclei of the trapezoid body.

306.3 GABA-LIKE IMMUNOREACTIVITY IN THE ANTEROVENTRAL COCHLEAR NUCLEUS. R. A. Altschuler, M. D. Oberdorfer, M. H. Parakkal*, K. A. Reeks*, J. M. Zempel* and R. J. Wenthold. Lab. Neuro-otolaryngology, NINCDS and NEI, NIH, Bethesda, MD. 20205 and Dept. of Neurophysiology, Univ. of Wisconsin, Madison, WI 53706

GABA is a major inhibitory neurotransmitter with a widespread distribution in the mammalian CNS. There is strong evidence supporting a neurotransmitter role for GABA in the cochlear nucleus, with reports suggesting that most GABA in the cochlear nucleus is associated with intrinsic neurons. To determine the morphology and distribution of GABAergic terminals in the anteroventral cochlear nucleus (AVCN) of the guinea pig, antibodies made against GABA were used in an immunocytochemical study. Antibodies were produced in rabbits against GABA conjugated to BSA. GABA specific antibodies were obtained by affinity purification and characterized by immunoblotting. Guinea pigs were perfused with 4% paraformaldehyde and 0.1% glutaraldehyde. Immunoperoxidase or immunogold staining procedures were performed on free-floating vibratome sections with primary antibody at dilutions of 1/1000 to 1/3000. GABA positive terminals were found throughout the AVCN. These terminals were lightly or densely packed with oval/pleomorphic vesicles and made symmetric contacts on cell bodies of stellate and spherical cells. Similar terminals were seen making axo-dendritic synapses in surrounding neuropil and a few contacts suggesting axo-axonal synapses. Terminals containing flattened or spherical vesicles were unstained. A few small cells with GABA immunoreactive staining were also observed in the AVCN. This study suggests that there is an extensive and complex GABAergic input to the guinea pig AVCN. GABA immunoreactivity seen in intrinsic neurons in the dorsal cochlear nucleus may be the source of some of this input.

306.4 EVIDENCE FOR A NEUROTRANSMITTER ROLE FOR GLYCINE IN THE GUINEA PIG COCHLEAR NUCLEUS. C.G. Staatz-Benson and S.J. Potashner. Dept. of Anatomy, University of Connecticut Health Center, Farmington, CT 06032.

Glycine has been proposed as a putative neurotransmitter in the mammalian cochlear nucleus (CN). Studies were performed to determine if mechanisms exist in the CN for the high-affinity uptake and the synaptic release of glycine. To measure uptake, the CN was rapidly dissected from anesthetized guinea pigs and divided into the anteroventral (AVCN), posteroventral (PVCN), and dorsal (DCN) cochlear nuclei. The segments were chopped (100 um slices), weighed, pre-incubated (30 min. 37°C), then incubated in Ringer with several concentrations of $^{3}$H-glycine. After 5 minutes, slices were separated from the medium by centrifugation, washed, and homogenized in acid. Each division of the CN accumulated $^{3}$H-glycine by a high-affinity uptake process which was sodium-dependent and temperature-dependent. Kinetic analysis demonstrated Km (uM) and Vmax (nmoles/g cell water/min) values of 34, 97 in the AVCN, 35, 93 in the PVCN and 36, 110 in the DCN. These Km and Vmax values are consistent with those for high-affinity uptake of glycine in other areas of the central nervous system.

To measure glycine release, dissected segments of the CN were first allowed to take up $^{14}$C-glycine. They were then superfused with low-sodium, glycine-free medium which was collected in 2 minute fractions. CN segments were then subjected to electrical field stimulation (2 ms duration, 200 Hz, 40 mA (AVCN); 100 Hz, 60 mA (PVCN and DCN)) for 4 minutes during the superfusion. Radioactivity was measured in the collected superfusate fractions and in the tissue at the end of the superfusion. The electrically-evoked release of $^{14}$C-glycine was quantified as the pSER value; the percent of tissue $^{14}$C-glycine released as a result of stimulation. pSER values were 5.6% in the AVCN, 8.7% in the PVCN and 6.0% in the DCN. Approximately 80-95% of this release was dependent on the presence of calcium in the medium, suggesting that this proportion of the release was from synaptic structures.

These findings suggest that each subdivision of the guinea pig CN contains mechanisms for the high-affinity uptake and electrically-evoked, calcium-dependent release of glycine. The presence of these presumed synaptic activities is consistent with a potential neurotransmitter role for glycine in the CN.

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306.5 THE PATTERN OF GAD-LIKE IMMUNOREACTIVE AXON TERMINALS ON IDENTIFIED CELL TYPES IN THE CAT VENTRAL COCHLEAR NUCLEUS. R.L. Saint Marie, D.K. Morest and C. Brandon. Dept. of Anatomy, Univ. Conn. Health Ctr., Farmington, CT 06032 and Dept. of Biological Chemistry and Structure, The Chicago Med. Sch., No. Chicago, IL 60064.

The distribution of GABAnergic axon terminals on identified cell types was examined histochemically using an antibody to glutamate decarboxylase (GAD). The immunoreaction appears in all subdivisions with an intensity proportional to the amount of neuropil present in each region; e.g., the most staining occurs rostrally and dorsally, decreases caudally and ventrally as far as the zone of bifurcations, and increases again in the posterior division. These gradients compare well with biochemical assays of GAD levels in the guinea pig (Wenthold & Morest, 1976).

Each cell type has a characteristic labeling pattern, based on the size, concentration, and distribution of GAD+ endings on its soma. Spherical bushy cell somata in the anterior division are typically contacted by many medium-sized (1-2μ) terminals, some of which are isolated, others are associated with tight clusters of many small (0.5-1μ) endings. This pattern is interrupted by large naked patches which correspond to the unreactive end-bulbs of Held. Also, there is a tendency for the medium-sized endings to gather at the base of dendrites. Globular bushy cells in the anterior division have a pattern similar to that of the spherical cells, but the clusters of immunoreactive terminals are less tightly packed, the naked patches are less well defined, and there is a greater proportion of the medium-sized endings (>1μ). Octopus cells in the octopus cell area have immunoreactive terminals that are more evenly distibuted than those on bushy cells, although these endings also tend to aggregate into small clusters or short rows. Generally, the size of the immunoreactive terminals on octopus cells is larger (1-2.5μ) and the packing density of these terminals is similar on both the dendrites and soma. Stellate and giant neurons also have distinctive labeling patterns, but several varieties have been seen; e.g., some have few endings on their somata. In general, the immunoreactive terminals are evenly distributed on the somata and proximal dendrites, although some cells in the anterior division show a preferential accumulation of labelled terminals at the bases of dendrites. Small cells have been seen which are contacted by some of the largest GAD+ terminals encountered in this nucleus (up to 3μ).

Several of the patterns of immunoreactive terminals in this study resemble those of non-cochlear axons projecting from the trapezoid body and the dorsal cochlear nucleus in Golgi material and correspond to synaptic endings with small pleomorphic vesicles and symmetric junctions.

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306.6 ANTEROVENTRAL COCHLEAR NUCLEUS OF THE MOUSE IN TISSUE CULTURE. M. R. Martin, J. Mazzetta*, R. A. Altschuler and K. Reeks* Laboratories of Neuro-otolaryngology and Neurophysiology, NINCDS, NIH, Bethesda, MD 20205

During the past decade the anteroventral cochlear nucleus (AVCN) has been the site of an intensive investigation into the nature of the transmitter of the auditory nerve. The cumulative evidence strongly implicates excitatory amino acids, with receptors for the three known classes being present. In recent years pharmacological studies have focused on the in vitro tissue slice preparation of the mouse. This preparation, however, has the disadvantage of having a low yield of data, or poor quality recording, and the inability to do patch clamp analysis of the receptors on the neuronal membrane. To overcome some of these limitations we have made an initial attempt to culture the neurons of this region.

Sagittal sections (600 μm) of the cochlear nucleus of 7 day old mice were prepared and the region anterior to the incoming auditory nerve fiber was cut off for use in preparing cultures. Using standard tissue culture techniques both explant and dissociated cultures were prepared. High glucose MEM with 5% horse serum on mouse feeder layers was used. After 12 days in culture the cells were fixed with 4% paraformaldehyde and stained for neuron-specific enolase-like immunoreactivity (NSE-IR). The avidin/biotin complex immunoperoxidase technique was used with a polyclonal antiserum to NSE.

A variety of neurons can be found in both explants and dissociated cultures. NSE-IR positive neuron somas are mostly in the range of 10 to 20 μm in diameter, but larger neurons (up to 40 μm) are also found. Dendrites were also commonly positive for NSE-IR stain. However, the smallest NSE-IR positive neurons (8-10 μm) typically appear spherical with no processes. A number of neurons also have an elongated fusiform shape. By far the majority of neurons are multipolar.

Although a number of neuron types are present, it is hoped that this preparation will lead to a better understanding of the synaptic physiology and pharmacology of this region.

306.7 STIMULUS DEPENDENT NEURAL CORRELATIONS BETWEEN TYPE II AND TYPE IV UNITS IN DORSAL COCHLEAR NUCLEUS. HF.Voigt, Dept. of Biomedical Engineering, Boston University, Boston, MA 02215 and E.D.Young, Dept. of Biomedical Engineering, Johns Hopkins University, Baltimore, MD 21205.

Type II units in the unanesthetized, decerebrate cat dorsal cochlear nucleus (DCN) are thought to provide at least some of the inhibitory input to DCN type IV units. Evidence supporting this claim was provided by a previous, single-electrode, cross-correlation study that indicated brief decreases in the firing probability of type IV units following the occurrences of action potentials in simultaneously recorded type II units (Voigt, HF and Young, ED, J.Neurophys. 44:76-97, 1980). One shortcoming of the single-electrode technique for recording unit pairs is the inability to resolve overlapping action potential waveshapes. This results in cross-correlograms with artifactual zero values in the vicinity of zero delay, and thus obscures the shape of the cross-correlogram near the origin where type II-type IV inhibitory correlations are expected. We report here results obtained from 57 type II-type IV pairs recorded simultaneously with two independently maneuvered microelectrodes. Cross-correlograms were computed from acoustically driven activity because type II units lack spontaneous activity. For 55 type II-type IV pairs we chose to use 60s tones; the frequency was set to the type II unit's best frequency (BF) and the level was chosen to be about 10,20 or 30 dB above the type II unit's threshold. We found, in agreement with the earlier study, asymmetrical, inhibitory troughs (ITs) in the cross-correlograms of 18/55 pairs indicating a brief decrease in type IV firing probability following type II action potentials. Nearly half (12/25) of the type II-type IV pairs encountered with BF differences less than 0.3 octaves showed signs of correlated activity. In contrast only 6/30 pairs with BF differences greater than 0.3 octaves had correlated activity. Cross-correlograms were obtained at two or more levels for 11 of the 18 IT-correlated pairs. For 7 of the 8 pairs that had BF differences less than 0.3 octaves, the size of the IT decreased with increased sound level. In contrast, the ITs of all three of the pairs that had BF differences greater than 0.3 octaves increased in size with higher sound level. Thus we find that the size of the IT in the cross-correlograms of type II-type IV pairs depends on both stimulus level and on BF differences. (Work supported by NIH.)

306.8 CIRCUITRY IN THE MOUSE DORSAL COCHLEAR NUCLEUS. J.A. Hirsch and D. Oertel. Dept. of Neurophysiology, University of Wisconsin, Madison, WI 53706.

Many, but not all, of the large cells in the dorsal cochlear nucleus (DCN) are directly excited by fibers of the auditory nerve. Anatomical evidence shows that most of these cells also receive inputs from granule cells and other neurons in the DCN and from neurons in the ventral cochlear nucleus (VCN). We have studied the neuronal circuitry of the DCN by recording intracellularly from cells in brain slices of the cochlear nuclei. Slices were made with a cut 200 to 300 μm below the pial surface. Some slices contained parts of the DCN, VCN, and the attached root of the auditory nerve, others contained only the DCN. The drugs used were applied in the bath.

All cells tested were excited by 1.0 mM glutamate (5 cells). Glutamate is without effect in the VCN (Oertel, Brain Res. 302: 213, 1984). These results are consistent with much literature which suggests that granule cells make excitatory connections in the dorsal rather than the ventral division and that the transmitter released by granule cells is glutamate.

Spontaneous IPSPs are continually recorded in the DCN. Shocks delivered to the auditory nerve evoke EPSPs followed by trains of IPSPs. For each of the 6 cells tested, 1.0 μM strychnine blocked both the spontaneous and evoked IPSPs; 0.01 to 0.1 mM levels of the GABA antagonists bicuculline (4 cells) and picrotoxin (2 cells) had no effect. All inhibitory synaptic responses we see, therefore, are probably glycinergic.

To distinguish between inputs arising in the VCN from those endogenous to the DCN, we recorded from slices containing only the DCN. Spontaneous IPSPs remained in the absence of the VCN. This result, together with our observation that cells in the DCN but not the VCN are spontaneously active in slices, suggests that spontaneous IPSPs arise from interneurons of the DCN. When we stimulated through electrodes placed on the surface of the DCN, the evoked synaptic potentials resembled the response to electrical stimulation of the auditory nerve and we were surprised.

This work was supported by grants NS 17590 and NS 12732 from the National Institutes of Health.

306.9 **DISTRIBUTION OF RECEPTORS IN THE MOUSE COCHLEAR NUCLEUS: AN AUTORADIOGRAPHIC STUDY.** A. Frostholm, C. Schultz* and A. Rotter. Department of Pharmacology, California College of Medicine, U.C. Irvine, Ca 92717.

Light microscopic autoradiography of radiolabeled ligands was used to describe the distribution of seven receptors in the dorsal and ventral regions of the mouse cochlear nucleus: Muscarinic ACh receptor ($^3$H-quinuclidinyl benzilate); histamine receptor ($^3$H-mepyramine); glycine receptor ($^3$H-strychnine); GABA receptor ($^3$H-muscimol); adenosine receptor ($^3$H-cyclohexyladenosine); benzodiazepine receptor ($^3$H-flunitrazepam); nicotinic ACh receptor ($^{125}$I-alpha-bungarotoxin). Slide mounted sections were incubated under optimal binding conditions and prepared for autoradiography by the method of Young and Kuhar (Brain. Res. 175:255,1979). Silver grains were counted in dark field photomicrographs of coronal sections through the cochlear nuclei.

Highest levels of **$^3$H-muscimol** binding were observed over the granule cell layer of the posterior ventral nucleus (PVCN) and over the pyramidal layer of the dorsal nucleus (DCN). Other regions of the nucleus were unlabeled. The most intense **$^3$H-strychnine** binding was observed over the deep region of the DCN, with slightly lower densities over the molecular and pyramidal layers. Dorsal regions of the PVCN were moderately labeled; occasional clusters of very high labeling were visible over more ventral regions. The granule cell layer was of low density. The deep region of the DCN was moderately labeled by **$^{125}$I-alpha-bungarotoxin;** small patches of extremely high grain density were also scattered throughout this region. The molecular and pyramidal layers were slightly less densely labeled. In the ventral nucleus, a reticulated cascade of very high grain density was observed over the medial area, with globular clusters of high density spreading out laterally. The granule cell layer was unlabeled. Moderate **$^3$H-quinuclidinyl benzilate** binding was seen over the molecular layer, possibly extending into the pyramidal layer, of the DCN. A thin band of moderate grain density was visible over the granule cell layer of the PVCN. All other regions were unlabeled. Moderate, diffuse **$^3$H-mepyramine** labeling was visible throughout the DCN, with slightly higher grain density over the molecular, and possibly the pyramidal layers, than over the deep region of the DCN. The granule cell layer was of low to moderate grain density, while the remainder of the ventral nucleus was unlabeled. Low to moderate levels of diffuse **$^3$H-flunitrazepam** binding were observed over all laminar regions of the DCN, except for the most caudal sections where the deep region was unlabeled. The granule cell layer was moderately labeled, with the remainder of the PVCN being covered by a diffuse pattern of low to moderate grain density. The most intense **$^3$H-cyclohexyladenosine** labeling was seen over the molecular layer, possibly extending into the pyramidal layer, of the DCN. The granule cell layer of the PVCN was also densely labeled. All other areas were of low grain density.
(Supported by USPHS Grant NS 18089).

306.10 POSTNATAL CHANGES IN SYNAPTIC CIRCUITRY AND ELECTRICAL PROPERTIES OF CELLS IN THE VENTRAL COCHLEAR NUCLEUS OF MICE. S.H. Wu* and D. Oertel. Dept. of Neurophysiology, Univ. of Wis., Madison, WI 53706.

In mice the external and middle ear cavities are closed for the first 8 days after birth. They are fully open only after about 12 postnatal days. When mice begin to hear, they respond only to low-frequency sounds (<10 kHz). At 16 to 18 days they show mature audiograms with threshold minima at about 20 kHz (review: Shnerson and Pujol, in The Auditory Psychobiology of the Mouse, ed. by J.F. Willott, Thomas, Springfield, pp. 395-425).

We have traced some of the electrophysiological changes associated with maturation by recording from brain slice preparations of the ventral cochlear nucleus (VCN) of mice of various ages. Cells in the VCN responded with excitatory postsynaptic potentials (EPSPs) to electrical stimulation of the auditory nerve in the youngest animals from which recordings were made, 4 days old. EPSPs from such young animals fatigued with repetitive stimulation at rates higher than 1/sec. Synaptic delays were long and variable (0.7 to 3 msec). In animals 6 to 9 days old, EPSPs could sustain rates of 10/sec. Their latencies were shorter (0.9 to 1.4 msec) and less variable. In addition, inhibitory postsynaptic potentials (IPSPs) often followed the EPSPs. Physiologically, therefore, excitatory synaptic connections are evident before the inhibitory synaptic connections. In animals 10 to 13 days old, synaptic responses to stimulation of the auditory nerve could support repetition rates of 200/sec. In adults, synaptic responses can follow at rates up to 630/sec.

The intrinsic electrophysiological properties of cells in the VCN also change as the animals mature. In adult animals, bushy and stellate cells can be distinguished on the basis of their intrinsic electrical properties (Wu and Oertel, J. Neuroscience 4:1577, 1984). The two cell types can be distinguished as early as 7days after birth. In cells that fire repetitively, the stellate cells, the first action potential of a train reaches a higher peak and it is narrower than later action potentials. If an inward calcium current contributes to the rising phase of action potentials, it is too weak to be detected even in the presence of 1 μM tetrodotoxin and 25 mM tetraethylammonium chloride. After 13 postnatal days the properties of cells are like those of the adult.

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306.11 EFFECTS OF STIMULUS REPETITION RATE ON ABR THRESHOLD AND AMPLITUDE IN YOUNG AND ADULT MONGOLIAN GERBILS. G.S. McCoy* and E.W Rubel. Dept. Otolaryngology, Univ. VA Med Ctr., Charlottesville, VA 22908

Increased stimulus repetition rate is associated with increased latency and decreased amplitude in the adult auditory brainstem response (ABR). While the effects of repetition rate on ABR latency have been studied during ontogeny, the effects of rate on ABR threshold and amplitude as a function of age have received little attention. In the present study, the effects of stimulus rate on the threshold and amplitude of ABR waveforms in young and adult animals were evaluated.

The ABR waveforms of 16 day old and adult Mongolian gerbils were evoked by click stimuli presented at rates ranging from 1 to 80/sec. Wave I and wave IV thresholds were determined at each of five rates. Amplitudes of waves I and IV were obtained at each of seven rates and three suprathreshold intensity levels (15, 40 and 65dB SL).

At low repetition rates, thresholds for 16 day old animals were 10-15dB higher than those for adults. In adult animals, repetition rate had no significant effect on thresholds of waves I and IV. In 16 day old animals, wave I threshold was not significantly affected by stimulus rate, whereas wave IV threshold increased significantly with increasing repetition rate. In adult animals, wave I amplitude did not vary with stimulus rate at 15 and 40 dB SL, but at 65dB SL amplitude decreased at rates above 20/sec. In 16 day old animals, wave I amplitude decreased with increasing repetition rate at all intensity levels. This finding suggests that low threshold auditory fibers are capable of following high repetition rates in adults but not in young animals. Slopes of wave IV rate-amplitude functions in adults were similar across intensity levels; however, the slope of such functions in 16 day old animals varied with intensity. Increased rate effects at high intensities in young animals may reflect the relatively greater fatigability of synaptic connections in young animals.

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306.12 SYNAPTIC POTENTIALS OF NEURONS RECORDED FROM THE CHINCHILLA LATERAL SUPERIOR OLIVARY NUCLEUS. D.M. Caspary, C.L. Faingold and K.P. Berry*. Dept. of Pharmacology, Southern Illinois University School of Medicine, Springfield, IL 62708.

Neurons located in lateral superior olivary (LSO) nucleus receive inputs from both ears and are thought to code for the localization of sound in space. These neurons receive ipsilateral input from the homolateral ventral cochlear nucleus (VCN), while their predominant contralateral input is from the opposite VCN via a secure synapse in the ipsilateral medial nucleus of the trapezoid body (MNTB) (Glendenning et al., J. Comp. Neurol., 232:261-285, 1985). LSO neurons typically are excited (E) by ipsilateral stimulation and this response is strongly suppressed during binaural stimulation. Contralateral stimulation alone does not evoke an excitatory response but is the presumed source of the observed binaural inhibition (I). Results from recent studies suggest that neurons in the MNTB may be the source of a glycinergic input to neurons in the LSO and may be responsible for the observed binaural inhibition (Moore and Caspary, J. Neurosci., 3:237-242, 1983). In the present study intracellular recordings were used to identify and characterize the synaptic potentials mediating the IE response. Intracellular recordings were obtained from 11 IE neurons in the ketamine-barbiturate anesthetized chinchilla. Only neurons displaying ipsilateral excitation and profound binaural inhibition were examined. Contralateral 3.2 msec tone-burst stimulation 30-50 dB above characteristic frequency (CF) threshold evoked large (5-12 mV) IPSPs with durations up to 7 msec. Suprathreshold ipsilateral stimulation at CF elicited sustained EPSPs with 1-3 action potentials arising from the plateau. Stimuli of equal intensity delivered at 0 msec interaural time delay (simultaneous binaural stimulation), rarely evoked action potentials while truncated IPSPs remained visible. In addition, reversal potentials and current-voltage relationships were obtained for some neurons. Preliminary data suggest linear current-voltage relationships with subthreshold current injection.

These findings provide the first evidence that the binaural inhibition observed for principal LSO neurons is mediated by an IPSP and that these are similar in character to IPSPs recorded from spinal cord neurons known to receive an inhibitory amino acid input. It is of interest that although observed IPSPs and EPSPs were of approximately equal amplitude and similar duration, simultaneous binaural stimulation resulted in a total inhibition of the excitatory response.

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306.13 THE QUANTITATIVE DISTRIBUTION OF $^3$H-STRYCHNINE BINDING IN THE LATERAL SUPERIOR OLIVARY NUCLEUS OF THE GERBIL. D.H. Sanes*, W.A. Geary II, and G.F. Wooten. Depts. Neurol. and Otolaryngol., Univ. VA Med Ctr., Charlottesville, VA 22908.

The lateral superior olivary nucleus (LSO) integrates excitatory inputs driven by one ear with inhibitory inputs driven by the other ear. Iontophoretic studies indicated that glycine mediates this inhibition (Moore and Caspary, 1983). We have examined the concentration and distribution of $^3$H-Strychnine ($^3$H-SN) binding sites in the LSO of adult gerbils to better understand the organization of the inhibitory projection.

The kinetic characteristics were determined by varying $^3$H-SN concentration, incubation time, and wash time. The kinetic constants, $K_d$=6.4 nM, $B_{max}$=15.8 fm/section, k+1=3.74 x $10^5$/M-s, and k-1=1.82 x $10^{-3}$/s, are comparable to those previously reported (Young and Snyder, 1973). Sections were preincubated in phosphate buffer, incubated in 10 nM $^3$H-SN or 10 nM $^3$H-SN plus $10^{-2}$ M for 20 mins, and washed for 10 secs. Sections were apposed to LKB film or emulsion coated coverslips and the autoradiographic images analyzed on a densitometer. Grain density was converted to ligand concentration using calibrated tritium standards (Geary and Wooten, 1983). Autoabsorption was corrected for by injecting animals with $^3$H-2-Deoxyglucose, extracting alternate sections in chloroform, and comparing the autoradiographic images (Geary and Wooten, in press).

The LSO has an S shape in the coronal plane, with the top of the S lying dorsomedial to the bottom. We have taken densitometric measurements in 3 regions of the nucleus for sections containing both total and non-specifically bound ligand (3 animals; 5 nuclei; 220 measurements). Autoabsorption in dorsomedial and ventral LSO (2 animals; 4 nuclei; 40 measurements) is 27% and 28%, respectively. Specifically bound $^3$H-SN concentrations are corrected for by these amounts.

The dorsomedial, central, and ventral regions have bound ligand concentrations of 215 ± 65 fmoles/mg tissue wet weight ($\bar{X}$±SD), 125 ± 37 fmoles/mg, and 71 ± 25 fmoles/mg, respectively. These measurements are consistent both with preliminary cell density measurements in gerbil showing an approximate twofold difference between the dorsomedial and ventral regions of LSO, and the density of terminals from the medial nucleus of the trapezoid body in the cat (Glendenning et al., 1985).
(Supported by NIH Grants NS15478 and 1F32 NS07462-01)

306.14 RETROGRADE TRANSPORT OF $^3$H-GABA FROM THE COCHLEAR NUCLEUS TO THE SUPERIOR OLIVE IN GUINEA PIG. E.-M. OSTAPOFF, D.K. MOREST AND S.J. POTASHNER. U. CONN. HEALTH CENTER, FARMINGTON CT 06032

Biochemical evidence suggests that some of the descending inputs to the cochlear nucleus use GABA as a neurotransmitter (Potashner et al., 1985). To test this hypothesis, 0.3-1.0 ul of $^3$H-GABA (80 uM SA. 80 Ci/mmol) was injected into the cochlear nucleus. After survival times of 15 min, 5, 10 and 16 hr, the brain stem was prepared for light microscopic autoradiography. After a 15 min survival, only glial cells in the cochlear nucleus and a few trapezoid fibers were labeled. After 5 hr survival, retrogradely labeled neuronal cell bodies and fibers appeared in the superior olivary complex. In the trapezoid body clusters of labeled cells were seen in the lateral nucleus, ipsilaterally, and in the ventral nucleus, bilaterally. Also there were labeled cells in the dorsal periolivary nucleus, ipsilaterally, and in the anterolateral periolivary nucleus and ventral nucleus of the lateral lemniscus, bilaterally. Large and small cells of several types were labeled. In the cochlear nucleus, round or elongated small neurons were labeled in all layers of the dorsal nucleus; no labeled cell bodies were observed in the ventral nucleus, but heavy glial and endothelial labeling made observations here difficult. Survival times of more than 5 hr resulted in non-specific labeling.

When gabaculine, a suicide substrate of GABA-T, was injected 1 hr before $^3$H-GABA, a similar, but more intense pattern of retrograde labeling occurred in the superior olive after 2 and 5 hr survivals.

The following evidence suggests that the cell labeling in the superior olive is due to the high affinity uptake of $^3$H-GABA by synaptic endings in the cochlear nucleus and the retrograde transport of label by GABA-ergic neurons. 1) Since the concentration of $^3$H-GABA used is near the Km for its high-affinity uptake in vitro, contributions from low-affinity uptake should be small. 2) The labeling in the cochlear nucleus and superior olive is selective for certain cells. 3) There was no sign of uptake and retrograde transport by fibers in the restiform body or cochlear nerve, even when the injection site encroached on these structures.

Experiments are in progress with lower concentrations of $^3$H-GABA and different survival times to define the cell types in the cochlear nucleus which may be GABA-ergic. Other experiments are aimed at elucidating the distribution of the extrinsic and intrinsic GABA-ergic projections to the cell surface of specific cell types in the cochlear nucleus.

This study provides evidence for a descending projection system for inhibitory feedback from the superior olive to the cochlear nucleus. (Supported by NIH grants NS14347 and NS19036.

306.15 CALCITONIN GENE-RELATED PEPTIDE IMMUNOREACTIVITY IN THE SUPERIOR OLIVARY COMPLEX OF CAT AND RAT: A SPECIFIC LABEL FOR THE LATERAL OLIVOCOCHLEAR SYSTEM. L. F. Schweitzer,* S. M. Lu,* D. Dawbarn,* and N. B. Cant. Department of Anatomy, Duke University Medical Center, Durham, NC 27710 and MRC Neurochemical Pharmacology Unit, Cambridge, England, CB2 2QH.

Two systems of axons project from the superior olivary complex (SOC) of the brain stem to the cochlea (Guinan, Warr, and Norris, JCN, '83). The lateral olivocochlear system comprises small axons that project mainly to the ipsilateral inner hair cell region. The medial system comprises larger axons that project mainly to the contralateral outer hair cell region. The two systems appear to arise from neurons lying in separate locations in the SOC. Further support for a distinction between the lateral and medial olivocochlear neurons in the SOC is provided by our finding that the lateral olivocochlear neurons are immunoreactive for calcitonin gene-related peptide (CGRP), a recently characterized peptide found in the CNS, whereas the medial neurons are not.

Cats and rats were anesthetized with pentobarbital and perfused through the heart with a PLP fixative (McLane and Nakane, J. Histochem. Cytochem., '74). Sections 50 µm thick were cut on a Vibratome. The sections were incubated in an antibody to CGRP (rabbit, Amersham) diluted 1:1000 or 1:2000. Immunocytochemistry was done with the ABC method (Hsu et al., J. Histochem. Cytochem., '81), and immunoreactive structures were labelled with diaminobenzidine.

In the SOC of the cat, immunoreactivity for CGRP is confined to cells in the dorsal hilus of the lateral superior olivary nucleus (LSO), to cells dorsal to the LSO, and to a few cells located along the margins of the nucleus. The cells are densely labelled as are their dendrites, which penetrate the substance of the LSO, particularly its lateral limb. No immunoreactive neurons are present in any other part of the SOC, but labelled axons and terminals can be traced into the lateral parts of the ventral cochlear nucleus. In the SOC of the rat, cells immunoreactive for CGRP are found within the LSO rather than around its margins, although not all LSO neurons are labelled. As in the cat no other cells in the SOC are labelled. The different distributions of label in the cat and rat parallel the differences between these species in the locations of lateral olivocochlear cells.

Although both lateral and medial olivocochlear neurons stain positively for acetylcholinesterase (Warr, JCN, '74), only the lateral neurons exhibit CGRP immunoreactivity. It may be possible to use the antibody to CGRP to distinguish between the terminals of the lateral and medial axons in the cochlea.

Supported by NIH.

306.16 MORPHOLOGICAL FEATURES OF FIVE NEURONAL TYPES IN THE GERBIL LATERAL SUPERIOR OLIVE. R.H. Helfert and I.R. Schwartz. Departments of Anatomy and Head & Neck Surgery, UCLA School of Medicine, Los Angeles, CA, 90024.

At least five morphologically distinct neuronal types exist within the neuropil of the gerbil lateral superior olive (LSO), excluding the hili. The features of four of these classes are nearly identical in morphology, orientation, and proportions to those found in the cat LSO, and can be classified using the feline terminology of principal (PR), multiplanar (MU), marginal (MA), and small (SM) neurons (Helfert and Schwartz, Neurosci. Absts. 10:844, 1984). The fifth cell type found in the gerbil is similar to the PR neuron, but receives significantly fewer axosomatic synapses. The distribution of this type is similar to that we observed for the gerbil acetylcholinesterase-positive (AChE) neurons.

The gerbil PR neuron is uniplanar and multipolar, oriented rostrocaudally in planes roughly perpendicular to the curvatures of the LSO. In transverse sections the perikarya of the PR cells appear fusiform and bipolar (mean dimensions: 22x10µm). More than 65% of the somata of PR neurons is apposed to synaptic terminals. PR neurons constitute about 70% of gerbil LSO neurons.

Approximately 10% of LSO neurons are MU neurons. They appear round or polygonal in transverse Nissl sections; appear multipolar in transverse Golgi sections; and show a pattern of synaptic input similar to that in PR neurons.

Approximately 5% of LSO neurons are of the MA type. MA neurons are fusiform and bipolar in transverse sections, and are oriented along the contours of the LSO perpendicular to PR neurons.

Another 10% of gerbil LSO neurons are SM cells. They are: either spherical or oval when seen in transverse sections; found throughout the LSO matrix; have a major axis rarely exceeding 10µm; and have less than 10% of their somatic surface in contact with synaptic terminals.

Several neurons with less than 50% of their somatic surfaces apposed to synaptic terminals have been identified in the medial and middle limbs. At the light microscopic level, this fifth neural type is similar to PR cells in both morphology and orientation. They are typically smaller than PR neurons, with average transverse dimensions of 17x7µm. About 5% of LSO neurons are of this type. AChE neurons are located throughout the rostrocaudal extent of the gerbil LSO. They are found in the dorsal hilus and, in contrast to the cat, within the medial and middle limbs and, to a much lesser extent, within the lateral limb. The similarity in size and distribution between the AChE neurons and the PR-like neurons with reduced axosomatic contacts suggest that they may be the same cells. EM studies required to prove this correspondence are in progress.

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306.17 CONVERGENCE OF BINAURAL PATHWAYS INVOLVING THE SUPERIOR OLIVARY COMPLEX AND THE DORSAL NUCLEUS OF THE LATERAL LEMNISCUS. Shneiderman, A. and C. K. Henkel. Department of Anatomy, Bowman Gray School of Medicine, Winston-Salem, NC 27103.

Convergence of binaural pathways to the inferior colliculus is important in neural mechanisms involved in sound localization. In this study we examined an indirect binaural pathway from the lateral (LSO) and medial (MSO) superior olivary nuclei to the inferior colliculus involving the dorsal nucleus of the lateral lemniscus (DNLL). Injections of tritiated leucine or WGA-HRP were placed in LSO, MSO or DNLL in cats and axonal projections were evaluated with light microscopy using autoradiographic and histochemical methods. Both olivary nuclei gave rise to projections to DNLL that were topographically ordered along the known frequency representation in the respective nuclei. In DNLL projections from MSO were ipsilateral and overlapped the regions of the bilateral projections from LSO. These projections appeared to arise as right angle collaterals of axons projecting to the inferior colliculus. Results of injections in DNLL showed that projections from DNLL to the inferior colliculus formed a banded pattern that was more distinct in the contralateral central nucleus than in the ipsilateral central nucleus. DNLL projections extended throughout the pars medialis, pars centralis and pars lateralis of the central nucleus and axons continued in the orientation of the bands into layers III and IV of the dorsal cortex of the inferior colliculus. DNLL also projected to the rostral pole nucleus of the inferior colliculus. Thus, DNLL projections share several similarities to binaural projections directly to inferior colliculus from LSO and MSO. DNLL projections are organized in bands with distinct interband spaces where there is less intense label as do LSO projections. DNLL projections also distribute to all subdivisions of the central nucleus where they may converge on cells with projections from the olivary nuclei and they also overlap olivary projections in the rostral pole nucleus of the inferior colliculus. In contrast to olivary pathways to the inferior colliculus, DNLL projections extend to the caudal third of the central nucleus and to the dorsal cortex where they do not converge with the projections of MSO or LSO. Further studies to define the structure of afferent bands in the inferior colliculus, the interrelationship of bands from specific binaural pathways, and the synaptic relationships of afferents to cells within the central nucleus should provide valuable information about the integration of binaural information in the inferior colliculus.

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306.18 LOW FREQUENCY RESPONSE PROPERTIES OF TRAPEZOID BODY FIBERS. G.A. Spirou*, W.E. Brownell, M. Zidanic* and P. Dulguerov* (SPON: W.A. Friedman). Depts. of Neuroscience and Otolaryngology, Univ. of Florida, Gainesville, FL, 32610.

Single axon recordings were made from the midline trapezoid body of adult cats under barbiturate anesthesia. Seventeen units of low CF were investigated for their rate-level and phase-level responses at high intensity. CF's of the units ranged from .48 to 4.38 kHz. Fourteen of 17 units exhibited phase shifts at high intensity between 90 and 230°. In most units (13 of 14) this was accompanied by a dip in the rate-level function between 25-90%. In two units in which tuning curves for the phase-rate shifts were obtained, minimum threshold occurred below CF. Generally, units were investigated at CF and 1 kHz. Only one unit, CF = .94 kHz, showed a phase-rate shift at CF but not at 1 kHz. Maximum driven rates at 1 kHz were often greater than those at CF, and ranged between 200 and 500 sp/sec. The 3 units not showing phase-rate changes may have had thresholds above the maximum SPL deliverable by our acoustic system, about 105 dBSPL. One large diameter fiber was labeled with HRP over a limited extent and showed the typical branching pattern of these units: the parent fiber continued rostral into the VNLL while the primary branch traveled in the direction of the MNTB. More completely filled axons of the same diameter and branching pattern deliver a calyx of Held into the MNTB.

Receptive fields of high frequency units (>10 kHz) were mapped. These units had low frequency tails which extended through the 1 kHz range. Frequently on/off PST's were found in the low frequency portion of the receptive field. An inhibitory surround 10-20 dB in extent was located under the excitatory tail. Slopes of rate-level functions at low frequency were enhanced at excitatory threshold as the units emerged from their inhibitory trough, and were greater than slopes of the rate-level functions at CF threshold. Changes in rate and temporal pattern of fiber discharge are two possible coding mechanisms for extending the dynamic range of the auditory system into the high intensity range at low frequencies. The presence of rate and phase-level shifts in these 2nd order fibers follows their description in the VIIIth nerve (Kiang et al. JASA 46:106 1969; Liberman and Kiang Hear. Res. 16:75-90 1984), and is further evidence for the security of the primary afferent to bushy cell synapse.

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306.19 ANATOMY AND PHYSIOLOGY OF OLIVOCOCHLEAR EFFERENT NEURONS IN THE GUINEA PIG COCHLEA. M. C. Brown. Research Laboratory of Electronics, M. I. T., and Eaton-Peabody Laboratory, Massachusetts Eye & Ear Infirmary, 243 Charles St., Boston, Ma. 02114.

Olivocochlear neurons in the superior olivary complex of the brainstem send axons to the cochlea to terminate in the organ of Corti, forming an olivocochlear efferent system. This report details the physiology and anatomy of olivocochlear neurons in the guinea pig cochlea at the single cell level.

Access to the spiral ganglion of the lower basal turn was created surgically in guinea pigs anesthetized with Nembutal and Innovar-Vet. Here, efferent fibers run in intraganglionic spiral bundles and were labeled with gross injections of horseradish peroxidase (HRP). Two fiber groups assumed to be olivocochlear efferents were distinguished based on their terminations in either inner or outer hair cell regions. Fibers terminating near inner hair cells were thin (< 0.7 um) and unmyelinated. Labeled efferents terminating on outer hair cells included thick myelinated and thin unmyelinated axons and terminated predominantly apical to the injection site.

Recordings of single units yielded presumed afferents with short latency responses (< 2.5 ms) to tone bursts at characteristic frequency (12 to 20 kHz) and irregular spike patterns. Presumed efferents responded with longer latencies (> 5 ms) to tone bursts and demonstrated regular spike patterns as has been reported previously (Fex, J., Acta Physiol. Scand., 55:S189, 1962; Robertson, D., Hear. Res., 15:113, 1984). Out of 51 "efferent" units, 65% were more sensitive to ipsilateral sound and 35% were more sensitive to contralateral sound. 83% gave excitatory responses to sound in one ear only with the remainder showing varying degress of sensitivity to sound presented in either ear. Most efferents were sharply tuned (characteristic frequencies 0.3 to 14 kHz), with tip sensitivities of 10 to 70 dB SPL. Two ipsilaterally excited efferents were intracellularly injected with HRP and yielded large, myelinated axons which branched to innervate 27 and 49 outer hair cells, respectively, over longitudinal extents of 0.5 and 0.66 mm. of the total cochlear length of 18 mm.

Thus the outer hair cell efferent fibers in the anesthetized guinea pig have frequency selective responses and at least in some cases, innervate relatively restricted regions of the receptor epithelium (equivalent to a quarter octave frequency span for afferents). The micropipettes used here are probably biased against the small axons of efferents associated with the inner hair cells, thus physiological data from this class of neurons is lacking. The functional properties of the outer hair cell efferents are consistent with a feedback system that is sharply tuned and which is in operation even in anesthetized animals.

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306.20 CENTRIFUGAL CHOLINERGIC PROJECTIONS TO SUBREGIONS OF CAT COCHLEAR NUCLEUS. D.A. Godfrey, K.L. Beranek*, L. Carlson*, J.D. Dunn and C.D. Ross, Depts. Physiol. and Anat., Oral Roberts Univ., Tulsa, OK 74171

To estimate what proportion of cholinergic elements in subregions of the cat cochlear nucleus derive from centrifugal pathways, choline acetyltransferase (ChAT) activities were mapped in both cochlear nuclei after placement of surgical cuts. Knife cuts were placed medial to the left cochlear nucleus by direct observation after exposure using a posterior fossa approach and aspiration of part of the cerebellar hemisphere. Seven to ten days later, cats were decapitated, brain pieces removed and rapidly frozen, 20 µm-thick sections cut at -20 ° C and freeze-dried. Sections of cochlear nucleus were microdissected into contiguous pieces which were weighed (about 1 µg) and placed into tubes for assay by a radiometric procedure. Data for 3 cats are shown below: one (Sham) in which the cochlear nucleus was exposed, but no cut placed, one (OCB cut) in which the cut penetrated the brain stem only slightly beyond the level of the olivocochlear bundle, and one (Complete cut) in which the cut penetrated to the ventral surface of the brain stem. Data for subregions are presented as lesion-side mean/control-side mean (%). AVCN, DCN, PVCN: anteroventral, dorsal, posteroventral cochlear nucleus. Differences statistically significant (t-test): x at p<0.05, + at p<0.01, * at p<0.001; ° no. samples insufficient for statistics.

| Region | Sham | OCB cut | | Complete cut | |
|---|---|---|---|---|---|
| AVCN rostral granular | 192/127(151) | 21/127 | (17)* | 23/254 | (9) ° |
| AVCN rostral | 44/49 (90) | 13/29 | (45)* | 9/44 | (21)* |
| AVCN caudal granular | | 54/97 | (56)x | 20/73 | (28)+ |
| AVCN caudal dorsal | | 22/28 | (79) | 6/21 | (30)* |
| AVCN caudal ventral | | 16/21 | (76) | 6/16 | (37)x |
| DCN molec. layer | 30/28 (109) | 28/36 | (77) | 16/31 | (51)* |
| DCN fus. soma layer | 46/44 (106) | 32/60 | (53)+ | 11/49 | (24)* |
| DCN deep layer | 20/17 (117) | 18/24 | (75) | 8/25 | (33)* |
| PVCN caudal | 8/6 (144) | 12/13 | (97) | 3/6 | (60)x |
| PVCN caudal granular | 41/36 (114) | 30/51 | (59) | 14/20 | (71) |

ChAT activities in almost all subregions of the cochlear nucleus were drastically reduced after virtually all its centrifugal innervation was cut. (Data from two other cats were in agreement with this result.) After OCB transection, significant reductions were found in rostral AVCN (in a second cat, the lesion/control % was 23) and its overlying granular region, and in the fusiform soma layer of the DCN. These results suggest that most cholinergic elements in the cat cochlear nucleus derive from its centrifugal innervation, and that branches from the olivocochlear system account for most of these elements in the rostralmost part of the nucleus. The molecular layer of the DCN apparently contains relatively more intrinsic cholinergic elements than most other subregions. (Supported by NIH Grant NS17176.)

306.21 LOCALIZATION AND LIGHT MICROSCOPIC CHARACTERIZATION OF NEURONS IN A REGION OF THE INFERIOR MEDULLARY VELUM ADJACENT TO THE COCHLEAR NUCLEI OF MAN. L. Terr. House Ear Institute, Los Angeles, CA 90057

The tenia of the inferior medullary velum is partly attached to the brain stem surface, including its area occupied by the cochlear nuclear complex. Since neurons of the complex -- particularly those in the dorsal cochlear nucleus (DCN) and caudal part of the ventral cochlear nucleus (VCN) -- may populate the most superficial layer of the brain stem, I examined the possibility of their penetration into the velum. Light microscopic examination of frontal and horizontal sections from postmortem human brains fixed in 4% formalin revealed a few neurons in the region of the tenia adjacent to the DCN surface. These neurons appeared in up to three groups rather than being scattered through the whole strip of the tenia adjacent to the DCN and VCN. The groups are located in the vicinity of the caudorosacral part of the DCN and the anterior boundary of the pontobulbar nucleus. The neurons within the tenia are located immediately under the extraventricular surface of the velum. They form round or oval-shaped regions, as in the organization of typical brain nuclei. The length of the short and long axes is 0.3 mm to 1.5 mm from it. The neurons most remote from the DCN surface in horizontal sections are approximately 1.5 mm. These neuronal groups are about 0.4 mm from the intraventricular surface of the tenia (ependyma). The thickness of the tenia in this region is about 0.7 mm. There are about 40 neurons in the largest area of the neurons accumulation in horizontal sections. The neurons are fusiform, pyramidal, multipolar, and round. There are no specific topographical distribution of these types of neurons in the tenia.

## REGIONAL LOCALIZATION OF RECEPTORS AND TRANSMITTERS III

307.1 AUTORADIOGRAPHIC LOCALIZATION OF INSULIN RECEPTORS IN RAT BRAIN: PROMINENCE IN OLFACTORY AND LIMBIC AREAS. J.M. Hill, M.A. Lesniak, J. Roth and C.B.Pert* (SPON: T. Insel). Section on Brain Biochemistry, Clinical Neuroscience Branch, National Institute of Mental Health, and Diabetes Branch, National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, Maryland 20205.

Both insulin and insulin receptors are widely but unevenly distributed throughout the central nervous system. While the role of insulin in the brain remains uncertain, insulin has been shown to modulate neuronal firing, stimulate the release of neurotransmitters, and influence food intake in several experimental systems.

In previous studies of insulin receptors in brain, membrane preparations from anatomically discrete regions have been used for localization. In the present study using $^{125}$I-insulin and frozen rat brain tissue under binding conditions optimized for ligand selectivity pattern and high signal to noise ratio (up to 95%) we have visualized the insulin receptor distribution pattern in rat brain with autoradiographic techniques.

By several criteria including specificity (for insulin-related ligands), the brain insulin receptors were qualitatively indistinguishable from insulin receptors previously characterized on brain and other more typical target tissues and distinct from receptors for the insulin-like growth factors. The $^{125}$I-insulin binding sites in brain formed a distinct pattern with high levels of binding in all olfactory areas and in closely related limbic regions. Binding was also prominent in the neocortex and the accessory motor areas of the basal ganglia and the cerebellum. Among ancillary brain structures only the choroid plexus demonstrated a high density of insulin receptors.

When insulin receptors were located on cortical or laminated structures the highest binding occurred in the superficial ("molecular" or "plexiform") layer, i.e. the layer which receives afferent input and/or is rich in the dentritic branches of principle neurons (e.g., external plexiform layer of the olfactory bulb and the molecular layer of the dentate gyrus).

The enrichment of insulin receptors in the olfactory and limbic systems, (a characteristic feature of other known neuropeptides), in addition to their prevalence dendritic fields suggests a neuromodulatory function for insulin in the brain.

307.2 DISTRIBUTIONS OF NICOTINIC AND MUSCARINIC CHOLINERGIC RECEPTORS IN MONKEY CEREBRAL CORTEX. J.B. O'Neill*[1], P.B.S. Clarke*[2], D.P. Friedman[1,3], and A. Pert[2], (SPON: R.M. Brown[3]). Laboratory of Neuropsychology[1] and Biological Psychiatry Branch[2], NIMH, Bethesda, MD 20205 and Neurosciences Research Branch[3], NIDA, Rockville, MD 20857.

The distributions of nicotinic (N) and muscarinic (M) cholinergic binding sites were compared in monkey cerebral cortex. 25 um thick, slide mounted, unfixed brain sections from two female rhesus monkeys were incubated to reveal either N or M binding sites as follows. For N sites, sections were incubated at room temperature for 20 min in pH 7.4, 50mM Tris-HCl, 8mM $CaCl_2$, and 1.7nM [$^3$H]L-nicotine. Slides were rinsed four times for 30 sec in the same buffer at 4°C and then quickly dried under a stream of cool air. Nonspecific binding was determined in the presence of 10uM L-nicotine bitartrate. For M sites, sections were incubated for two hrs at room temperature in pH 7.4, 0.01M PBS, with 0.6nM [$^3$H]Quinuclidinyl benzilate (QNB). They were then washed three times for five min in the same PBS buffer and dried as above. Nonspecific binding was determined in the presence of 1uM atropine sulfate. The sections incubated in [$^3$H]nicotine were apposed to tritium sensitive LKB film for either five or seven months. The sections incubated in [$^3$H]QNB were apposed to the LKB film for five weeks.

Both N and M receptors were found in all cortical regions, but M receptors were more widely distributed across the layers of a given field and had a wider variety of laminar labeling patterns across different fields than N receptors. N receptors were restricted to a band in layer III. This band was densest and widest in the primary sensory areas (A1, S1, and V1), but no other variations in pattern were apparent. The most common pattern for M receptors consisted of a dense band of label extending from layer I through upper layer III, a receptor poor band in lower layer III, and another band of receptors, less dense than the supragranular band, in the lower layers. Other laminar patterns of M receptors ranged from an apparently homogenous distribution across all the cortical layers in orbital frontal cortex, to variations of the bilaminar pattern described above in S1 and V1, to a single dense band in layer III of A1. Only in A1 did the N and M patterns appear similar.

The laminar patterns of distribution of both N and M receptors described here for the monkey differ from those desribed previously in the rat. Nonetheless, in both species, N receptors are located in either layers III or IV, the targets for incoming afferent fibers, suggesting a role for nicotinic regulation of this input. M receptors, by contrast, are densest in the upper and lower cortical layers, a distribution consistent with a role for muscarinic modulation of intracortical processing.

307.3 DISTRIBUTIONS OF NICOTINIC AND MUSCARINIC CHOLINERGIC RECEPTORS IN MONKEY THALAMUS. D.P. Friedman[1,3], P.B.S. Clarke*[2], J.B. O'Neill*[1], and A. Pert[2]. Laboratory of Neuropsychology[1] and Biological Psychiatry Branch[2], NIMH, Bethesda, MD 20205 and Neurosciences Research Branch[3], NIDA, Rockville, MD 20857.

The distributions of nicotinic and muscarinic binding sites were compared in the thalamus of the monkey. 25um thick, slide mounted, unfixed sections from two female rhesus monkey brains were incubated to reveal either nicotinic or muscarinic binding sites as follows. For nicotinic sites, sections were incubated at room temperature for 20 min in pH 7.4, 50mM Tris HCl, 8mM $CaCl_2$, and 1.7nM [$^3$H]L-nicotine. Slides were rinsed four times for 30 sec in the same buffer at 4°C and then dried quickly under a stream of cool air. Nonspecific binding was determined in the presence of 10uM L-nicotine bitartrate. For muscarinic sites, sections were incubated for two hrs at room temperature in pH 7.4, 0.01M PBS, with 0.6nM [$^3$H]Quinuclidinyl benzilate (QNB). They were then washed three times for five min in the same PBS buffer and dried as above. Nonspecific binding was determined in the presence of luM atropine sulfate. The sections incubated in [$^3$H]nicotine were apposed to tritium sensitive LKB film for either five or seven months. The sections incubated in [$^3$H]QNB were apposed to the LKB film for five weeks.

Nicotinic receptors were densest in all three anterior thalamic nuclei (AM, AV, and AD). In addition, both subdivisions of the medial dorsal n. (MDmc and MDpc), the lateral dorsal n. (LD), the lateral posterior n. (LP), and pulvinar complex contained moderately dense labeling. The lateral (LG) and medial (MG) geniculate nuclei and the enitre ventral group contained somewhat less label, and within this group the least heavily labeled nuclei were those of the ventrobasal complex (VPL and VPM). By contrast, the habenular nuclei, the intralaminar nuclei, and the midline nuclei were conspicous by their lack of label. The thalamic reticular n. contained moderately dense label.

Muscarinic receptors, like nicotinic receptors, were dense in the anterior nuclei, but in MD, the parvocellular division was more densely labeled than the magnocellular division, which contained little label. The pulvinar and LD also contained moderately dense labeling, but LP, like the entire ventral group, was less densely labeled. LG and MG again contained moderate amounts of label and the intralaminar nuclei again had little, if any, label. The nuclei of the midline, on the other hand, had a moderate amount of label, as did the lateral habenula, which was not labeled by [$^3$H]nicotine. The thalamic reticular n. contained muscarinic as well as nicotinic receptors.

307.4 DIRECT AUTORADIOGRAPHIC DETERMINATION OF MUSCARINIC M1 AND M2 RECEPTOR SUB-TYPE DISTRIBUTION IN THE RAT BRAIN: RELATIVE BINDING PATTERNS IN CHOLINERGIC NUCLEI AND PROJECTION AREAS. D.G. Spencer, Jr., E. Horvath* and J. Traber*, Neurobiology Dept., Troponwerke, Neurather Ring 1, 5000 Cologne 80, FRG.

The autoradiographic distribution of (3H)-oxotremorine-M and (3H)-pirenzepine binding to rat brain M1 and M2 receptors, respectively, was studied in order to evaluate the hypothesis that M2 receptors are good markers for cholinergic cell bodies and tracts, as opposed to M1 receptors which appear to label post-synaptic fields (Potter et al., TIPS suppl., 22-31, 1983). The binding of 1 nM (3H)-oxotremorine-M to brain slices was highly specific and saturable. Structurally unrelated muscarinic receptor ligands such as scopolamine, carbachol, and arecoline produced complete competition at 1, 10, and 100 uM, respectively. Regional distribution of M2 binding was quite different from that of M1 binding (labelled by 1 nM (3H)-pirenzepine): M2 receptors were found in brain-stem cholinergic nuclei (e.g., cranial nerve nu., parabrachial nu., dorsal and laterodorsal tegmental nu., trapezoid body nu., raphe nu., central gray, interpeduncular nu., and pontine nu.), inferior and superior colliculus, several thalamic areas (e.g., ant. pretectal area and anterodorsal, anteromedial, anteroventral, lateral posterior, posterior paraventricular, reticular, reuniens, and rhomboid nu.), hypothalamic regions, forebrain cholinergic nuclei (e.g., medial septum, diagonal band horiz. and vert. limb nu., nu. basalis, substantia innomenata), caudate-putamen, CA2 and CA3 of hippocampus, amygdala (primarily the basolateral nu.), olfactory bulbs and tubercle, cingulate gyrus, perirhinal cortex, and layers III and V of cerebral cortex; M1 receptors were found only in telencephalic structures such as layers I and II of the cerebral cortex (frontal, parietal, and striate cortex), caudate-putamen, nu. accumbens, olfactory tubercle, CA1 and dentate gyrus of the hippocampal formation, and basolateral nu. of amygdala. This pattern of M1 and M2 binding supports the idea that M2 receptors are closely associated with cholinergic cell nuclei and projection areas. However, M1 receptors can only be detected in telencephalic cholinergic terminal regions; thalamic and collicular terminal zones contained solely M2 receptors. Scatchard analysis of (3H)-oxotremorine-M slice binding in the inferior colliculus revealed one high-affinity site (r = -0.95), with a Kd of 1.88 and a Bmax of 1.42 pmol/mg protein. M2 receptor densities (fM/mg protein) as measured by slice binding had the following rank orders: superior colliculus (243), caudate-putamen (225), medial septum and diagonal band nu. (164) = frontal cortex (163), hippocampus (121), and cerebellum (53). 1 mM N-ethylmaleimide uncouples receptors of many neurotransmitters, thus reducing their ligand affinity. In the present experiments, it reduced the affinity of M2 receptors for (3H)-oxotremorine-M without altering their regional distribution in brain; M1 receptor distribution and affinity for (3H)-pirenzepine were unaffected by this treatment (in agreement with the receptor binding experiments of Flynn & Potter, Proc. Natl. Acad. Sci. 82:580-583, 1985). Taken together, these data strongly support the hypothesis that M1 and M2 receptors are not simply intraconvertable states of a single receptor protein, but may be anatomically and functionally distinct muscarinic receptor sub-types.

307.5 IN VIVO QUANTITATION OF FREE MUSCARINIC RECEPTOR DENSITY: EFFECTS OF DFP AND OF ATROPINE. K. A. Frey, B. Ciliax, D. Wieland* and B. W. Agranoff. Department of Neurology, Division of Nuclear Medicine and Neuroscience Laboratories, The University of Michigan, Ann Arbor, MI 48109.

Previous work from our laboratory has validated the use of [$^3$H]scopolamine and tracer kinetic analysis of a compartmental ligand distribution model for the measurement of muscarinic receptor binding in vivo (Frey et al., Soc. Neurosci. Abstract, 1984). In the present work, a simplification of the previous algorithm is derived which permits direct calculation of regional free receptor density from tracer accumulation in tissue at a single time point following IV bolus injection. Application of this simplified expression further requires knowledge of the regional blood-brain barrier permeability-surface area product for the tracer, the local rate of cerebral blood flow, and the integrated arterial plasma tracer concentration over the experimental period. Male Sprague Dawley rats were injected intravenously with 100 µCi of [$^3$H]scopolamine (specific activity 80-90 Ci/mmol), a continuous withdrawal of arterial blood for 90 min was begun, and 25-50 µCi of [$^{123}$I]HIPDM (Kung et al., J. Nucl. Med. 29:66-72, 1983) were injected IV. Following an additional 45 sec distribution period, the animals were killed and brain regions were counted for [$^{123}$I], and following its decay, for [$^3$H]. Local rates of blood flow were calculated from the [$^{123}$I]-HIPDM uptake using the indicator fractionation method (Van Uitert and Levy, Stroke 9:67-72, 1978). Regional free receptor densities were determined from tissue and plasma [$^3$H]scopolamine levels, the calculated blood flow, and the known blood-brain barrier permeability coefficient (Frey et al., ibid, 1984). In control animals, the simplified method yielded free receptor densities which were in good agreement with our previous results: highest in neocortex, followed by striatum and hippocampus. The cerebellum had 5-fold fewer free receptors than neocortex. Pretreatment of rats with a saturating dose of atropine sulfate (50 mg/kg IV) reduced the free receptor density to 10% of control in all regions studied. Pretreatment with the cholinesterase inhibitor DFP (2 mg/kg IM) reduced the free receptor densities in neocortex and striatum, but did not effect cerebellar binding. These results are consistent with the hypothesis that the free receptor density measured by tracer kinetic in vivo binding techniques is determined by both the total regional receptor concentration and synaptic neurotransmitter levels. The in vivo receptor binding method may thus be useful in detection of alterations in presynaptic activity as well as in receptor numbers.

307.6 IN VIVO AND IN VITRO LABELING OF $^{125}$I-ALPHA-BUNGAROTOXIN SUPRACHIASMATIC BINDING SITES - A QUANTITATIVE EVALUATION OF DISTRIBUTION. M.M. Miller and R.B. Billiar*, Dept. of Obstetrics and Gynecology, Royal Victoria Hospital, McGill University, Montreal, Quebec H3A 1A1.

The distribution of $^{125}$I alpha-bungarotoxin (α-BTX), a putative nicotinic cholinergic receptor ligand was studied both in vitro and in vivo in the suprachiasmatic nucleus (SCN) of the rat hypothalamus. For in vitro studies 20 µ frozen frontal sections containing SCN were incubated with either varying concentrations of radioligand or unlabeled α-BTX and tissues processed for light microscopic autoradiography using NTB-2 and 8 days of development. Areas and perimeters of cresyl-violet-stained sections were measured using a Bioquant Analysis System and grain counts and distributions determined. We observed a distinctive and specific labeling pattern of the SCN. Grains tended to localize diffusely and uniformly in more rostral regions, but clustered densely in the dorsal and lateral mid-SCN, and dorsally in the mid-caudal SCN; in the most caudal regions no labeling was observed. Labeling patterns remained the same regardless of concentration of radioligand. Cold competition controls did not demonstrate grain counts above background levels. For in vivo investigations third ventricular infusion of either $^{125}$Iα-BTX, or unlabeled α-BTX with $^{125}$Iα-BTX was performed, and 24 hours later animals were perfused percardially (glutaraldehyde-paraformaldehyde) and hypothalamic tissues processed. One µserial plastic sections of SCN were incubated with NTB-2 for 47 days. No less than 70 micrographs from each of 4 regions (rostral, mid-rostral, mid, and mid-caudal) were examined in a blind study. Areas and perimeters of 1601 plastic-embedded cells were measured (Bioquant); the mean area of all cells in 360 micrographs from the rostral to caudal extent of the SCN was 71±23 um$^2$. Cells ranged in size from 55±19 um$^2$ (rostral-central) to 93±23 um$^2$ (mid-dorsal). In the rostral SCN, where cells were relatively small (range 55±19 um$^2$ - 83±8 um$^2$), $^{125}$I grains were distributed uniformly (from 0.003-0.005 grains (g)/um$^2$; Σ15±3 - 19±3g/hpf). In the mid-SCN, grains were distributed most densely laterally (.015g/um$^2$; Σ44.2±7 g/hpf), dorsally (.013 g/um$^2$; Σ54±6 g/hpf), and dorsal-laterally (.012 g/um$^2$; Σ44±14 g/hpf), while ventrally labeling was less dense (.007 g/um$^2$; Σ26±6 g/hpf). Cells in these sectors measured 93.3±22, 88.6±30, 74.5±20, and 65±25 um$^2$ respectively. The area of the SCN containing the larger neurons is morphologically different from the rest of the nucleus and receives a substantial number of afferent projections while sending dendrites laterally to the adjoining anterior hypothalamic area or dorsally into the periventricular region. Localization of $^{125}$Iα-BTX within the neuropil of these particular areas of the SCN may indicate a different functional interaction between these cells and those of adjacent areas either within or outside of the SCN. Supported by NIHR1HD94314.

307.7 REGIONAL DISTRIBUTION OF MUSCARINIC CHOLINERGIC BINDING IN TWO SPECIES OF WILD SONGBIRD AS DETERMINED BY TRITIUM-SENSITIVE FILM AUTORADIOGRAPHY. G.F. Ball*, B. Nock, B.S. McEwen, and J.C. Wingfield*. (SPON: P. Marler). The Rockefeller University, New York, N.Y. 10021

Seasonally breeding birds show dramatic changes in the sensitivity of their neuroendocrine systems to light and other environmental stimuli during the reproductive cycle. Recent work involving the blood sampling of free living breeding wild birds has correlated changes in pituitary gonadatropin secretion and gonadal steroid secretion with a variety of naturally occurring stimuli including not only daylength, but also behavioral interactions, weather, and the phenologic progression of spring.

As a prelude to functional studies of the neuroendocrine integration of this myriad of environmental information we have begun mapping, using quantitative autoradiography, the distribution of neurotransmitter receptor binding sites that may be involved in the regulation of the hypothalamo-hypophyseal-gonadal axis. Subjects consist of males from two species of songbird: the European Starling, Sturnus vulgaris, and the Song Sparrow, Melospiza melodia both of which have been extensively studied in the wild. Adult animals were decapitated, and their brains were rapidly removed, frozen on dry ice, and stored at -80°C. Coronal brain sections (25u) were cut and thaw-mounted onto subbed slides. Muscarinic receptors were labelled using ($^3$H) scopolamine, a muscarinic antagonist. Slices were exposed to tritium sensitive LKB film for 1 week. Autoradiograms were analyzed using a computer assisted densitometer.

In both species heaviest binding occurred in certain structures in the paleostriatal complex especially the paleostriatum augmentatum (PA), the lobus parolfactorius (LPO), and Area X. Binding was moderate in the hyperstriatum ventrale and in the optic tectum and low to moderate in areas of the hypothalamus. Overall patterns are quite consistent between the two species. These findings agree well with in vivo autoradiography performed in Zebra Finches by Ryan and Arnold (J.Comp. Neur. 202:211 1981) and with investigations on mammals which have found the greatest amount of binding in the caudate-putamen and the nucleus accumbens, areas thought to be homologous with PA and LPO-Area X, respectively.

307.8 CHARACTERIZATION AND REGIONAL DISTRIBUTION OF ADENOSINE DEAMINASE ACTIVITY IN RAT BRAIN. J.D. GEIGER and J.I. NAGY. Dept. of Pharmacology, Univ. of Manitoba, Winnipeg, MAN. R3E 0W3.

Specific neural systems utilizing adenosine as a neurotransmitter/neuromodulator have yet to be identified. One possible approach to such an identification in rat brain may be localizing neural tracts immunoreactive for adenosine deaminase (ADA), a major degradative enzyme for adenosine (Nagy et al., Science 224:166, 1984), and characterizing adenosine transport sites labelled with [$^3$H]nitrobenzylthioinosine ([$^3$H]NBI) (Geiger et al., J. Neurosci. 5:735, 1985; Geiger and Nagy, Brain Res. Bull., 13:657, 1984). The regional distribution patterns of neural systems immunoreactive for ADA correspond closely with that of adenosine transport sites (Nagy et al., Neurosci. Lett. in press). In order to quantitatively assess this correspondence we have investigated the characteristics and regional distribution of ADA in rat brain.

ADA activity was measured by a HPLC technique modified from that of Hartwick et al. (J. Chromatogr. Sci. 16:427, 1978). Enzyme activity was optimal over a pH range of 7.0 to 8.0 and was linear with protein (brain homogenates) concentrations of 0.1 to 1.6 mg. Using 0.3 mg protein and 500 μM adenosine, the amount of product formed was linear for incubations up to 60 min. The $K_m$ and Vmax values for whole brain were 95.2 μM and 64.2 nmoles product formed (hypoxanthine + inosine)/mg protein/30 min. Of the 40 brain regions examined the five areas containing the highest levels of enzyme activity (listed in decreasing order) were olfactory bulbs, posterior hypothalamus, superior colliculus, medulla oblongata and olfactory cortex. ADA activity in olfactory bulbs was 9 times higher than frontal cortex, the area with the lowest activity. The distribution pattern of adenosine transport sites appears to parallel that of ADA-immunoreactive fibers in brain areas lacking cell bodies immunoreactive for ADA. A poor correlation was noted between levels of [$^3$H]NBI sites and ADA activity in brain structures containing ADA-immunoreactive neuronal parykarya. (Supported by the Medical Research Council of Canada, Health Sciences Centre Research Foundation and Manitoba Health Research Council).

307.9 PRESUMPTIVE GLUTAMERGIC/ASPARTERGIC AFFERENTS TO THE VENTRAL STRIATO-PALLIDAL REGION. T.A. Fuller, F.T. Russchen* and J.L. Price. Depts of Psychiatry, Anatomy & Neurobiology, Washington Univ Sch Med, St. Louis, MO 63110.

Recent biochemical evidence has suggested a substantial glutamergic/aspartergic (Glu/Asp) input to the ventral striato-pallidal region in the rat, but the sources of these afferents have been only grossly localized to neocortex and allocortex (Walaas, Neurosci 6:399; Davies et al, Neurosci Lett 45:105). To investigate this, we have used $^3$H-D-aspartate ($^3$H-D-Asp) as a "neurotransmitter specific" retrograde tracer for Glu/Asp projections (Streit, J Comp Neurol 191:429) and wheat germ agglutinated-horseradish peroxidase (WGA-HRP) as a nonspecific retrograde tracer for all projections. Injections of $^3$H-D-Asp or WGA-HRP were made into the ventral pallidum, ventral striatum or olfactory tubercle, and after 18-48 hr the brains were fixed and prepared for autoradiography or HRP histochemistry.

The results show that injections of $^3$H-D-Asp label specific subsets of the 'total' afferents labeled by comparable WGA-HRP injections. Unlike WGA-HRP, $^3$H-D-Asp did not label the dopaminergic ventral tegmental area or substantia nigra or the noradrenergic locus coeruleus, and only an occasional $^3$H-D-Asp labeled cell was found in the serotonergic dorsal raphe. However, many cells and fibers in cortical and non-cortical areas are labeled by transport of $^3$H-D-Asp. The heaviest presumptive Glu/Asp inputs to the ventral pallidal area arise in the midline and medial intralaminar thalamic nuclei and in the amygdala (particularly the central and basomedial nuclei and the nucleus of the lateral olfactory tract (NLOT)). Fibers to the ventral striatal region arise in the medial frontal cortex, agranular insular cortex, olfactory cortex, olfactory tubercle, as well as in the midline and intralaminar thalamic nuclei, and the amygdala (especially the lateral and basolateral nuclei and the NLOT). Finally presumptive Glu/Asp inputs to the olfactory tubercle arise in agranular insular (rostral aspect) and olfactory cortices, the midline and intralaminar thalamic nuclei, the amygdala (especially the basolateral and basomedial nuclei and the NLOT) and the lateral supramammillary nucleus. The projections from the NLOT to the ventral striatum and the olfactory tubercle are bilateral, and there are also contralateral inputs to the ventral striatum from the agranular insular cortex and the basolateral amygdaloid nucleus. Based upon morphology and cell size, the thalamic cells labeled by injection of $^3$H-D-Asp into these three regions appear to be somewhat different although overlapping populations.

This indicates that specific subsets of afferent neurons to the ventral striato-pallidal area take up and retrogradely transport $^3$H-D-Asp and therefore are presumptively glutamergic and/or aspartergic. (Supported by RSDA MH00330 and NIH NS-0918.)

307.10 THREE-DIMENSIONAL AUTORADIOGRAPHIC LOCALIZATION OF QUENCH-CORRECTED GLYCINE RECEPTOR SPECIFIC ACTIVITY IN THE MOUSE BRAIN USING $^3$H-STRYCHNINE. W.F. White and A. Wang. Depts. of Neurosci., Harvard Med. Sch. and Children's Hosp., Boston, MA 02115.

Diffusable substance autoradiographic techniques have been widely employed for the localization of neurotransmitter receptors in the CNS. Current methodologies suffer from inadequate quantification of receptor specific activity from single sections through three-dimensional morphological regions and differential quench by the tissue. These limitations were addressed here by determining the three-dimensional distribution of $^3$H-strychnine binding site specific activity from quench-corrected autoradiographic data. The analyses were performed using a VAX 11-780 computer and a Megatek 7295 3-D color raster display in the Image Graphics Lab. at Children's Hosp. The brains from C57BL/6J mice were serially sectioned at 10 μm from the cervical cord to the frontal pole. Sections separated by 120 μm were preincubated for one hour in 100 mM NaCl, 50 mM $NaPO_4$, pH 7.4, incubated one hour in 5 nM $^3$H-strychnine, washed in four changes of fresh buffer for a total of one min., dipped in distilled water, and dried with anhydrous air. Autoradiograms were prepared by exposing the sections to LKB Ultrofilm for four weeks. The density of exposed silver grains was quantified using a scanning densitometer (Optronics P-1000) at a resolution of 50 μm, and following background subtraction, converted to specific activity. Differential quench was determined by incubating adjacent sections in $^{125}$I-Bolton and Hunter reagent or $^3$H-succinimidyl proprionate according to the methods of Herkenham and Sokoloff (Brain Res. 321:363-368). The optical densities of autoradiograms prepared from these sections were determined and registered with the data sections. The normalized ratio of optical density between the iodinated and proprionated sections was used to correct quench on a pixel by pixel basis. Data sections were registered into three-dimensional images of binding site distribution. Receptor density was visually represented by color density coding and displayed either as solid mosaics or as isodensity contour lines. These data demonstrate a marked regional distribution of $^3$H-strychnine binding sites in the mouse CNS. Specific binding was confined to gray matter areas with a marked rostro-caudal distribution. The highest levels of binding were seen in the substantia gelatinosa and ventral horns of the spinal cord, the spinal nucleus of cranial nerve V, the hypoglossal nucleus and dorsal motor nucleus of cranial nerve X. Moderate binding was observed in the remaining grey matter of the cord, numerous nuclear groups of the medulla and brainstem, and the corpus quadrigemini. Low levels of binding were found in the thalamus and hypothalamus. Both the cerebral and cerebellar cortices lacked specific binding. These studies demonstrate the usefulness of powerful computer methodologies for the localization of specific neurotransmitter function.

307.11 BENZODIAZEPINE RECEPTOR LABELING IN VIVO WITH [$^3$H]-Ro 15-1788. M.J. Kuhar and N.E. Goeders. Department of Neuroscience, Johns Hopkins University School of Medicine and the Laboratory of Neuroscience, NIDA Addiction Research Center, Baltimore, MD 21205.

The ability to study benzodiazepine (BZD) receptors in vivo can result in many useful applications. Imaging these receptors by PET scanning and other techniques such as autoradiography may provide insight as to the mechanism of action of these drugs and as to the organization and function of various brain regions. However, some reports of the in vivo labeling of brain BZD receptors (Chang and Snyder, Eur. J. Pharm., 48, 213, 1978; Williamson et al., Life Sci., 23, 1935, 1978) are actually "ex vivo" techniques because the tissues were removed, homogenized and washed to reduce nonspecific binding. This investigation was designed to provide a more desirable approach where BZD receptors are labeled in the tissues of intact animals.

Adult male ICR mice (30 to 35 g) were injected via the tail vein with 167 uCi/kg of [$^3$H]-flunitrazepam ([$^3$H]-Flu), [$^3$H]-methyl-clonazepam ([$^3$H]-Clo) or [$^3$H]-Ro 15-1788 and were sacrificed at various time points. For determinations of non-specific binding, mice were pretreated with clonazepam (5 mg/kg, i.p.) 30 minutes prior to the intravenous injection. The brains were rapidly removed and dissected into cerebral cortex, cerebellum and medulla to determine the regional distribution of the ligands at the various time points. In vivo binding was also determined in the presence of a number of peripheral and central-type BZD receptor ligands to investigate the pharmacological specificity of the binding. The saturability of ligand accummulation in the cerebral cortex was also examined.

Of the three drugs tested, only [$^3$H]-Ro 15-1788 resulted in workable specific/nonspecific binding ratios. The binding was specific and was only displaced by central-type BZD receptor ligands. Increasing concentrations of [$^3$H]-Ro 15-1788 resulted in a saturation of available receptors. In addition, the autoradiographic distribution of BZD receptors by the in vivo labeling procedure was similar to that found by in vitro labeling with [$^3$H]-Ro 15-1788. The results of this investigation suggest that Ro 15-1788 is a useful ligand for studying BZD receptors in vivo. (Supported by USPHS Grants DA 00266, MH 00053, NS 15080 and MH 09111 and grants from the Bristol-Myers Company and the McKnight Foundation).

307.12 IMMUNOCYTOCHEMICAL EVIDENCE FOR THE COLOCALIZATION OF $GABA_A$ AND BENZODIAZEPINE RECEPTORS IN DEFINED GABAERGIC SYNAPSES OF THE RETINA. H. Möhler*, J.G. Richards, W. Haefely and J.Y. Wu. Pharm. Res. Dept., F. Hoffmann-La Roche & Co., Ltd., CH-4002 Basel, Switzerland and Dept. of Cell Biol., Baylor College of Medicine, Houston, Texas 77030.

Previous immunohistochemical studies with antibodies to glutamate decarboxylase(GAD) (Wu, J.Y. et al., Mol. Cell. Biochem. 39: 229, 1981) have revealed horizontal cells and a subpopulation of amacrine cells in the vertebrate retina to be GABAergic. Processes of these interneurons arborize in the inner plexiform layer where they form the majority of their synaptic contacts with other amacrine cells and, to a lesser extent, with bipolar and ganglion cell processes. In accordance with these observations and with the proposed GABA-potentiating mechanism of action of benzodiazepines(BZs) in the CNS is the finding that $GABA_A$ and BZ receptors are also observed in the inner plexiform layer after radiolabelling with $^3$H-Ro 15-1788 and $^3$H-muscimol in vitro. Retinal BZ receptors appear to have the binding characteristics (e.g. pharmacological specificity, GABA-shift) of neuronal receptors.

Using monoclonal antibodies(mAb) to the $GABA_A$/benzodiazepine receptor complex isolated from bovine cerebral cortex (Schoch, P. et al., Nature 314:168, 1985), it has now been possible to identify the receptors in defined GABAergic synapses of the rat retina. Receptor antigenic sites were localized immunohistochemically(PAP method) in four distinct bands in the inner plexiform layer and around cells with the characteristic shape and location of amacrine cells. Adjacent sections stained with GAD mAb revealed a similar distribution in the inner plexiform layer. Moreover, ultrastructural studies revealed that the immune reaction for the BZ receptor was exclusively present in synaptic contacts.

The functional role of GABAergic synapses in the transmission between photoreceptors and bipolar and ganglion cells in the retina is not known. BZs attenuate the light-induced increase in dopamine turnover in a subpopulation of amacrine cells, which would be in line with the presence of receptor antigenicity on a small proportion of these cells. Whether therapeutic concentrations of BZs affect retinal function, however, is not clear. The fact that changes in visual perception do not seem to have been observed with BZs in man might indicate that ongoing GABAergic inhibition is usually close to maximal and, therefore, not potentiated by BZs.

307.13 VISUALIZING Kd and Bmax: FLUNITRAZEPAM BINDING PARAMETERS. A.W. Toga, E.M. Santori*, and R.C. Collins. Laboratory of Neuro Imaging, Department of Neurology and Neurosurgery and The McDonnell Center for the Study of Higher Brain Functions, Washington University, St. Louis, MO. 63110.

Autoradiographic receptor binding has improved the study of receptors because of the visual nature of the primary data that is generated. In the analysis of binding kinetics this visual component is lost. The goal of this study was to use current techniques of digital image analysis to visualize Bmax and Kd of benzodiazepine receptors in rat brain.

Fresh rat brain, along with two brain paste filled fiducial columns of PE 205 tubing were freeze-mounted onto an object holder. A blocking apparatus fixed the orientation of the brain and set the fiducial columns orthogonal to the object holder. The brain and two pieces of tubing were embedded in a gelatin matrix.

Flunitrazepam (FLU) binding was assayed utilizing the procedures developed by Young and Kuhar (JPET 212:337-346, 1980). Total binding was determined in triplicate at five FLU concentrations. Nonspecific binding was determined in the presence of 1 uM clonazepan, but only at the two highest FLU concentrations. Sections were processed for quantitative autoradiography.

Autoradiographic data was transformed to concentrations of radioactivity and of each set of triplicates digitally averaged. All arithmetic operations were performed on perfectly aligned images. Image alignment computations were performed using coordinates provided by the fiducials and required no user interaction. Nonspecific binding (NSB) within grey structures is linearly related to the concentrations of FLU. By interpolating a line through the two average NSB binding images and zero, the amount of NSB at any concentration of FLU could be estimated. New specific binding images were generated by subtracting the concentration-dependent amount of NSB from the five total binding images. Each specific binding image was then divided by its respective FLU concentration. Eadie-Hofstee analysis (linear regression of B vs B/F) was performed for all pixels of the image. The slope of the regressions produced an image of Kd. An image of Bmax was generated using the y-intercept. With this procedure inter and intra animal alterations in Kd and Bmax are now amenable to visual survey.

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307.14 RECEPTORS FOR NERVE GROWTH FACTOR IN THE RAT CENTRAL NERVOUS SYSTEM. R.J. Riopelle, P.M. Richardson and V.M.K. Verge* (SPON: S.K. Ludwin). Department of Medicine (Neurology), Queen's University, Kingston, Ont. K7L 3N6, and Division of Neurosurgery, McGill University, Montreal, Quebec H3G 1A4.

The regional distribution and biochemical properties of receptors for nerve growth factor (NGF) in the rat central nervous system (CNS) were investigated by receptor autoradiography and steady state binding analysis.

For radioautography, cryostat sections were incubated with $^{125}$I-NGF at 40pM with or without unlabelled NGF (40 nm). In the spinal cord, specific labelling was maximal in layers I, II, and III (Rexed) of the dorsal horn. Much of the binding was abolished by previous avulsion of appropriate dorsal roots, and therefore presumed to be on primary sensory afferents. In the brain, $^{125}$I-NGF bound specifically in the basal forebrain, hippocampus, and other discrete areas.

Plasma membrane fractions were prepared from dorsal spinal cord and several regions of the brain for steady state binding studies. $^{125}$I-NGF bound at high affinity ($K_D$ 20 pM) to membranes from the dorsal spinal cord, hippocampus, and basal forebrain, but not the cerebellum. Lower affinity sites were found in all regions. Radioautographs of SDS gels of solublized affinity-labelled {1-ethyl-3-(3-dimethylaminopropyl) carbodiimide} fractions of spinal cord and basal forebrain revealed one major band of molecular weight greater than 100 Kd. This band was not seen in the presence of excess unlabelled NGF.

For primary sensory and septohippocampal neurons, the results confirm and extend previous observations on specific uptake and retrograde axonal transport of NGF. The functions of NGF-like molecules on responsive neurons during development, maintenance, and repair in the CNS remain to be established. (Supported by MRC Canada and MS Society Canada).

308.1 OSMOTIC STIMULATION OF VASOPRESSIN RELEASE FROM PERIFUSED HYPOTHALAMO-NEUROHYPOPHYSEAL EXPLANTS. C.D. Sladek and R.W. Clough. Departments of Neurology and Anatomy, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642.

Vasopressin (VP) release in vivo and from hypothalamo-neurohypophyseal (HNS) explants maintained in static culture is stimulated by increased osmolality of the extracellular fluid or culture medium. A perifusion culture system has been utilized to evaluate the time course of this response. HNS explants were prepared from male rats (125-150 gms) and placed individually in 0.5 ml perifusion chambers (Endotronics). Explants were perifused at 1-2 ml/hr with $F_{12}$ culture medium supplemented with 20% fetal calf serum, 1 mg/ml glucose, and penicillin, streptomyocin, and bacitracin. Effluent from each chamber was collected in 10 or 15 min fractions and VP concentration was measured by radioimmunoassay. Following an 18 hr equilibration period, the effect of various osmotic challenges (NaCl) on VP release were evaluated in two experiments.

In the first experiment, 5 explants were exposed to 2 NaCl challenges 3 hours apart which resulted in increments of 15 and 42 mOsmol/kg $H_2O$ respectively. The increases occurred over 45 mintues and were sustained for an additional 30-45 minutes. There was a significant increase in VP release associated with both challenges ($p<.05$) and the larger increase in osmolality resulted in a greater release of VP. However, the response to the increase in osmolality was limited to the initial 30 minutes of the challenge and was not sustained throughout the period of elevated osmolality.

A second experiment was performed to evaluate the relationship between the rate of change in osmolality and VP release. Four explants were exposed sequentially to 3 pulses of NaCl separated by 50 minute washout periods. In all 3, osmolality of the perifusate increased from 292 to 330 mOsmol/kg $H_2O$. This occurred in 10, 30 and 40 minutes in the 3 pulses respectively and the increase was sustained following the third pulse. The absolute increase in VP release was comparable for all 3 pulses; however, it was achieved more slowly during the third pulse and was not sustained during static hypertonicity.

These preliminary observations suggest that the osmosensitive mechanisms endogenous to the HNS explant are sensitive to changes in osmolality, rather than static elevations in osmolality.

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308.2 $\alpha_1$-ADRENORECEPTOR-MEDIATED RELEASE OF ARGININE VASOPRESSIN (AVP) AND OXYTOCIN (OXY) FROM PERFUSED RAT HYPOTHALAMO-NEUROHYPOPHYSIAL EXPLANTS. M.F. Mazurek*, J.C.R. Randle, D. Kneifel* and L.P. Renaud. Dept. of Neurology, Massachusetts Gen. Hosp. Boston, MA. 02114 and Neurosciences Unit, Montreal Gen. Hosp. Research Institute, Montreal, Canada H3G 1A4.

The prominent noradrenergic innervation of neurosecretory neurons in the hypothalamic supraoptic and paraventricular nuclei suggests an important role for norepinephrine (NE) in regulating AVP and OXY release from the neurohypophysis. Recent electrophysiological data indicate that electrical stimulation of the brainstem A1 and A2 cell groups, where most of these fibers originate, can increase the firing of neurosecretory neurons. Similar results follow exogenous application of NE and alpha agonists. The present in-vitro experiments examined the influence of NE on AVP and OXY release.

Perfused explants of rat hypothalamus were prepared as described previously (Bourque & Renaud, J. Neurosci. Methods 7:203, 1983). In addition, both internal carotid arteries were cannulated to obtain optimal perfusion of the entire explant, and bovine serum albumin (0.1%) and phenol red (10 mg/l) were added to the perfusion medium. The pars distalis was carefully removed, leaving only the neurointermediate lobe attached, enabling easier diffusion of secretory products into the medium. A suction pipette was positioned immediately caudal to the pituitary and medium was collected via a peristaltic pump. Following a 1 hr preincubation, 1 min samples were collected throughout 1 hr experiments. AVP and OXY were measured using highly sensitive radioimmunoassays characterized by HPLC and serial dilution curves. Drugs were added directly to the perfusion medium for periods of 5 to 10 minutes at 20 minute intervals.

NE induced a prompt, sustained and reversible increase in the release of AVP from basal levels of less than 2 pg/min to 45 ± 13 pg/min (mean ± S.E.M) at $10^{-5}$M NE, and >100 pg/min at $10^{-4}$ M NE. Phenylephrine (PHE) ($10^{-4}$M) also stimulated AVP release to 31 ± 10 pg/min. NE elicited a parallel but more modest release of OXY, with values rising from basal levels of <1 pg/min.to 2.6 ± 0.2 at $10^{-5}$M NE, and 16 ± 11 pg/min at $10^{-4}$ M NE. The release of both AVP and OXY in response to $10^{-5}$M NE was virtually abolished in the presence of 5 x $10^{-8}$M prazosin. These concentrations of NE and PHE that increase immunoreactive AVP and OXY release are identical to those that enhance the excitability and promote bursting activity among SON neurons in this preparation (Randle et al., Brain Res. 307: 374, 1984).

These data support the interpretation that the endogenous noradrenergic innervation to hypothalamic neurosecretory neurons is facilitatory to efficient hormone release and mediated by alpha-1 adrenoreceptors. (Supported by the MRC and the FRSQ).

308.3 NEUROPEPTIDE Y AND ENDOGENOUS NORADRENALINE STIMULATE SECRETION OF VASOPRESSIN IN THE RAT. J.O. WILLOUGHBY AND W.W. BLESSING. Centre for Neuroscience and Department of Medicine, Flinders University of South Australia, Bedford Park, South Australia 5042.

Vasopressin-secreting neurohypophyseal neurons are densely innervated by processes of A1 noradrenaline neurons located in the ventrolateral medulla. Neuropeptide Y (NPY) is co-localized in A1 cells and is present in terminals in the supraoptic nucleus (Everitt et al, Neurosci. 11, 443-462 1984). Injection of noradrenaline into the supraoptic nucleus causes dose-dependent vasopressin secretion by activating alpha-1 adrenoceptors (Willoughby et al Neurosci. Lett. Supp 19 S41 1985). This study examines whether NPY releases vasopressin when injected into the supraoptic nucleus and whether endogenous noradrenaline, released by local injections of tyramine, does likewise.

Male Porton rats (250-350 g) were prepared with a chronic indwelling right atrial catheter and bilateral intracerebral guide cannulae directed at the supraoptic nuclei (Paxinos and Watson, bregma -1.3, lateral ± 2 mm). One week later, 1.2 ml blood samples for vasopressin radioimmunoassay were taken from unanesthetized animals before, and 5 and 20 minutes after bilateral injections into the supraoptic nucleus, with 30 guage needles inserted 9 mm below brain surface, through the guide cannulae. The plasma was removed and the red cells, resuspended in saline, were re-injected after the next sample. Agents were administered in 0.25 ul of Ringer's solution. Accuracy of injection sites was histologically confirmed.

Vasopressin responses (mean±SEM) are shown in the table:

| Agent | vasopressin (pg/ml) 5 min | 20 min |
|---|---|---|
| Ringer's (n=8) | 16±5 | 10±2 |
| Noradrenaline (10 nmol, 14) | 118±13 ** | 17±2 ** |
| NPY (1.0 nmol, 6) | 85±18 ** | 52±10 ** |
| Tyramine (100 nmol, 6) | 57±10 * | 24±5 * |

Significantly different from Ringer's * $P<0.05$, ** $<0.01$.

The results indicate that endogenous noradrenaline, released from nerve terminals in the supraoptic nucleus, stimulates vasopressin secretion. In addition there was a prompt and sustained response to NPY. Both noradrenaline and NPY may be co-transmitters participating in the baroreceptor-initiated secretion of vasopressin which is mediated by A1 neurons (Blessing and Willoughby, J. Physiol., in press).

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308.4 REGIONAL DISTRIBUTION OF MUSCARINIC AND PUTATIVE NICOTINIC CHOLINERGIC RECEPTORS WITHIN THE RAT HYPOTHALAMO-NEUROHYPOPHYSEAL SYSTEM. K.M. Michels*, R.B. Meeker* and J.N. Hayward. Dept. Neurology and Neurobiology Program, Univ. North Carolina, Chapel Hill, N.C. 27514.

Mechanisms for muscarinic cholinergic (mACh) and nicotinic cholinergic (nACh) stimulation of vasopressin (VP) and oxytocin (OT) release are present in the basal hypothalamo-neurohypophyseal system (HNS) (Barker et al, 1971; Gregg, 1985; Nordmann et al,1971; Sladek, 1983). The specific anatomical site(s) of action of mACh and nACh agonists in HNS are not fully described. Utilizing $^3$H-quinuclidinylbenzilate ($^3$H-QNB) as a probe for the muscarinic cholinergic receptors (mAChR) and using $^{125}$I-$\alpha$bungarotoxin ($^{125}$I-$\alpha$ BTX) as a probe for the nicotinic cholinergic receptors (nAChR), we have verified the presence of high affinity mAChR and nAChR in different regions of HNS. Our new technique for quantitative assessment of binding to fixed tissue sections ($^3$H-QNB with 1% acrolein; $^{125}$I-$\alpha$BTX with 0.5% paraformaldehyde) allowed pharmacological characterization of the receptor, autoradiographic localization and immunohistochemical staining on the same section. A single high affinity ($K_D$ = 8 x $10^{-11}$ M) site was labeled by $^3$H-QNB in the rat hypothalamus and neural lobe with a density of 140-340 fmoles/mg protein. Silver grains from $^3$H-QNB were of moderate density over the neural lobe, of low density throughout the hypothalamus but absent within the supraoptic nucleus (NSO). There was no clear relationship between $^3$H-QNB binding and VP and OT cells within the hypothalamus. In contrast, $^{125}$I-$\alpha$BTX binds to high ($K_D$ = 4 x $10^{-11}$ M) affinity sites in the rat HNS with a density of only 21 fmoles/mg protein. These $^{125}$I-$\alpha$BTX high affinity binding sites are concentrated in the NSO and the nucleus circularis (NC). The density of silver grains is equally distributed over VP and OT immunoreactive neurons and processes in both NSO and NC. We find little or no detectable binding of $^{125}$I-$\alpha$BTX in the neural lobe of the rat. These data demonstrate a regional anatomical distribution of cholinergic receptors within the rat HNS, with putative nicotinic cholinergic receptors (nAChR) localized on the membranes of magnocellular (MgC) neurons in the hypothalamus and muscarinic cholinergic receptors (mAChR) localized on the membranes of MgC neuron terminals in the neural lobe. Furthermore, we find that the nAChR are distributed at several sites on the membranes of VP and OT MgC neurons within the NSO and NC of rat hypothalamus. These data suggest a differential cholinergic regulation of VP and OT release: nAChR at soma-dendritic MgC sites in the hypothalamus and mAChR at axonal terminal MgC sites in the neural lobe.

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308.5 MORPHOMETRY OF CAPILLARIES IN RAT NEURAL LOBE AND SUPRAOPTIC NUCLEI. P.M. Gross, N.M. Sposito*, S.E. Nornes*, A.B. Butler and J.D. Fenstermacher. Department of Neurological Surgery, SUNY at Stony Brook, NY 11794-8122.

Capillaries of the neural lobe (NL) are the site of secretion of vasopressin originating from magnocellular neurons in the hypothalamic supraoptic nuclei (SON). The capillary bed of NL is exceptionally dense and the rate of blood flow in NL is extremely high (about 5.7 ml/g/min compared to 1.5 ml/g/min in cerebral grey matter structures such as SON). Using light and electron microscopic morphometry, we studied and compared the capillary beds and ultrastructure of endothelial cells in these two neurosecretory structures. The brains and pituitary glands of adult Sprague-Dawley rats were perfusion-fixed with 2% paraformaldehyde and 2% glutaraldehyde in 0.1 M phosphate buffer. Two micron-thick sections of NL and SON were obtained and stained with toluidine blue for light microscopy; ultrathin sections were taken and stained with uranyl acetate and lead citrate for electron microscopy. Results are presented as the mean ± SD for NL and SON capillaries measured in the number of rats shown in parentheses. *$p < 0.05$.

| | Neural Lobe | Supraoptic Nuclei |
|---|---|---|
| Capillary Bed | | |
| Density (#/mm$^2$) | 1014±51 (6) | 1038±126 (3) |
| Volume (%) | 3.2±0.3 | 3.1±0.2 |
| Surface area (mm$^2$/mm$^3$) | 32.5±2.3 | 29.8±1.8 |
| Endothelial Cells | | |
| Mitochondria (#/micron$^2$) | 0.36±0.11*(3) | 1.41±0.31 (3) |
| Junctions (#/micron$^2$) | 0.45±0.04 | 0.57±0.09 |
| Vesicles (#/micron$^2$) | 11.5±2.5* | 6.2±0.7 |
| Fenestrations (#/cross section) | 5±0.7* | none |

The findings demonstrate that the capillary beds of NL and SON have similar characteristics for density and volume as evaluated from light micrographs but are dissimilar morphologically as assessed by quantitative electron microscopy. The fewer endothelial mitochondria, greater number of endothelial vesicles and fenestrations, and extraordinary surface area and blood flow in capillaries of NL likely explain its high permeability to circulating solutes, i.e., its "absence" of a blood-brain barrier.

308.6 A LIBRARY OF MONOCLONAL ANTIBODIES WHICH BIND TO THE RAT NEUROHYPOPHYSIS. H.J. Cranston, M.J. Durick*, S.J. Hapner and C.M. Paden. Dept. of Biology, Montana State Univ., Bozeman, MT 59717.

Monoclonal antibodies (MAbs) which recognize antigens of the neuronal cell surface are proving to be powerful probes of developmental and functional processes in a variety of systems. In an attempt to produce MAbs against surface antigens of hypothalamic neurosecretory cells, we have employed membranes from fixed rat neurointermediate lobe as immunogens. Because the majority of the volume of the neural lobe consists of axons of neurosecretory cells, this tissue provides a naturally enriched source of neurosecretory cell membranes. Six MAbs which bind to fixed sections of rat neurohypophysis have been obtained to date.

The neurointermediate lobe was dissected from unperfused, decapitated Holtzman rats and post-fixed by immersion in Zamboni's overnight. A crude membrane pellet was obtained following rinsing and homogenization in hypotonic tris buffer. 100 g of protein from this pellet was used as an immunogen in in vitro activations of splenocytes obtained from naive Balb C BYJ mice. Following incubation for 3-5 days, fusions with P-3/X-63-Ag 8.65 myeloma partners were performed using minor modifications of standard protocols. When hybridoma growth became apparent, initial screening for antibody secretion was done using a modified ELISA procedure which detects mouse immunoglobulins in the culture media. Further immunocytochemical screening of spent media from positive wells was then performed on cryostat sections of fixed rat pituitary by indirect immunofluorescence. Antibody producing hybridomas were cloned twice by limiting dilution, and large quantities of MAbs produced by induction of ascites tumors.

Five of the 6 MAbs exhibit different staining patterns on whole pituitary sections. MAb F3-10 stains only the neural lobe in a diffuse manner; MAb 5B is also specific to the neural lobe, but staining is punctate over a diffuse background. MAb 1F binds primarily to the intermediate lobe in a punctate manner, with only faint reactivity present in the neural lobe. MAbs 11A and 7B both exhibit strong punctate staining in neural lobe with fainter staining visible in the anterior lobe as well, while MAb 5G stains anterior and neural lobes equally in a diffuse manner. Although raised against adult tissue, all 6 MAbs also bind to neonatal pituitary.

These results indicate that the rat neurointermediate lobe contains various proteins which are immunogenic in the mouse. While it is not possible to determine whether MAb binding is to neurosecretory or pituicyte processes at the light microscope level, further characterization of binding at the EM level and to sections of hypothalamus is in progress.

This research was supported by a Grant-In-Aid from the Montana Heart Association and by NSF BNS-8408061.

308.7 PURIFICATION OF FIXED NEUROPHYSIN-CONTAINING HYPOTHALAMIC NEURONS BY FLUORESCENCE ACTIVATED CELL SORTING. S.J. Hapner, D. Berglund*, C.J. Welsh*, G.S. Stuart* and C.M. Paden. Dept. of Biology, Montana State Univ., Bozeman, MT 59717.

Fluorescence activated cell sorting (FACS) offers the potential for cytometric analysis and purification of identified neuronal populations labeled by specific fluorescent markers. However, application of flow cytometry to viable neurons is currently limited by a scarcity of cell-type-specific surface markers. Purification of fixed cells using readily available antibodies to intracellular molecules is an alternative approach which could lead to production of new cell-surface antibodies by using the isolated fixed cells as immunogens (P.A. St. John and J.L. Barker, Soc. Neurosci. Abst. 9: 7, 1983). As a step toward this goal, we have succeeded in using FACS to isolate significant numbers of a small neuronal population, the neurophysin-containing neurons of the supraoptic (SON) and paraventricular (PVN) nuclei.

SON and PVN were dissociated from slabs of fresh 8-day-old rat hypothalami using a Palkovits punch. Following mincing and fixation in Zamboni's for 3 hr, cells were incubated with collagenase at 37° C for 30 min, then transferred to Ca free PBS containing 1 mM Mg, 1% BSA, 0.25 M sucrose, 0.1% Tween 20 and DNAase. Dissociation was done by successive trituration through hypodermic needles of decreasing bore (18-25g). Debris was removed by settling and passage through a 44μ nylon mesh. Cells were incubated for 1 hr in a 1:1000 dilution of rabbit anti-rat neurophysins (gift of Dr. Allan Robinson) followed by fluorescein conjugated goat anti-rabbit IgG (Cappel) at 1:100 for 1 hr. Flow cytometry and cell sorting were performed on a Becton-Dickinson FACS 440 using either flourescence or forward scatter triggering and log mode fluorescence analysis. A distinct peak of highly fluorescent cells representing 1.7% of the total population was isolated. This peak was absent from both cerebral cortical cells treated as above and from SON-PVN cells when normal rabbit serum was substituted for anti-neurophysins. Fluorescent microscopy of sorted cells indicated a purity of 85-90%, yielding an enrichment of 50 fold. About 2000 cells were obtained per brain, with morphology varying from round to bipolar with attached processes.

It appears that sufficient numbers of relatively rare neurons can be obtained in this manner for use as in vitro immunogens. In addition, this technique could be used to rapidly examine large numbers of fixed cells for the colocalization of multiple antigens using the dual fluorescence capabilities of the FACS.

This research was supported by a Grant-In-Aid from the Montana Heart Association and by NSF BNS-8408061.

308.8 OXYTOCIN INHIBITS ACTH, EPINEPHRINE, AND NOREPINEPHRINE SECRETION IN THE URETHANE-ANESTHETIZED RAT. D.M. Gibbs, Dept. of Repro. Med., UCSD, La Jolla, CA 92093.

Oxytocin (OT) is a hypothalamic hormone which generally has a stimulatory effect on ACTH secretion both in vitro and in vivo. As part of a study of ACTH-releasing factors in hypophysial portal blood, we tested the effects of iv OT administration on plasma ACTH levels in urethane-anesthetized rats. Surprisingly, iv injection of 10 mcg OT lowered plasma ACTH levels by about 35% ($p<0.01$) and 1 mcg OT lowered ACTH levels by about 13% ($p<0.05$) whereas the similar peptide arginine vasopressin (AVP) had no effect. The 10 mcg dose of OT produced peak OT levels of 340 ng/ml 1 minute after injection and 30 ng/ml 10 minutes after injection. Because of the very high baseline levels of ACTH, epinephrine (EPI), and norepinephrine (NE) in the urethane-anesthetized rat, and because of the involvement of beta-adrenergic pituitary receptors in the stimulation of ACTH secretion, we reasoned that the paradoxical inhibition of ACTH secretion by OT might be mediated by inhibition of peripheral catecholamine secretion. Measurement of plasma catecholamines before and after iv administration of 10 mcg OT revealed a 53% inhibition of EPI ($p<0.01$) and a 43% inhibition of NE ($p<0.05$). Administration of the beta-adrenergic antagonist propanolol (400 mcg) 15 minutes before the beginning of the experiment completely blocked the inhibitory effects of OT on ACTH secretion and in fact unmasked the stimulatory effects of OT normally seen in conscious animals and in vitro.

To determine the site at which OT acts to inhibit catecholamine secretion, bisected rat adrenal glands were superfused with oxygenated Medium 199 at 37 C. Adrenals exposed to $10^{-6}$M OT for 10 minutes secreted more than 30% less EPI and NE than control adrenals suggesting that the inhibition of EPI and NE secretion by OT in vivo occurs, at least in part, directly at the level of the adrenal. AVP had no effect on in vitro catecholamine secretion.

These data support the hypothesis that peripheral catecholamines may at times be directly involved in the control of ACTH secretion. In addition, these experiments suggest that OT, which has recently been identified in the adrenal medulla (Ang, V.T.Y., and Jenkins, J.S, JCEM 58:688, 1984), may have important paracrine functions in the regulation of adrenal catecholamine secretion.

This work was supported in part by NIH grant AM-32517 and a Mellon Foundation Faculty Scholar Award.

308.9 GnRH RELEASE FROM THE MEDIOBASAL HYPOTHALAMUS (MBH): IN VITRO REGULATION BY OXYTOCIN. M. Gambacciani*, S.S.C. Yen*, D.D. Rasmussen, Dept. of Reproductive Medicine, UCSD, La Jolla, CA 92093.

Both suckling and stress stimulate oxytocin (OT) and inhibit LH secretion in the rat, suggesting that a functional interaction may exist between these endocrine responses. Since OT and GnRH are both localized in hypothalamic neuronal systems with terminals in the external zone of the median eminence (ME), we hypothesized that OT may suppress LH secretion by inhibiting hypothalamic GnRH release. Accordingly, an in vitro incubation system was used to evaluate GnRH release from adult male rat MBHs in response to OT. MBHs were removed following decapitation and incubated at 37°C for 12 min in 0.45 ml of oxygenated Medium 199 containing 0, 0.1, 10 or 1000 pM OT (n=9/group). These media were then replaced by identical media plus 56 mM KCl, and the incubation was continued for 4 min. GnRH release was determined by radioimmunoassay of media from the 12 min (basal) and 4 min ($K^+$ stimulated) incubations. Mean (±SE) basal GnRH release was 9.1±1.1 pg/12 min in the control group; OT treated MBHs released 6.6±0.8 (0.1 pM), 4.5±0.4 (10 pM) and 3.6±0.3 (1000 pM) pg/12 min. $K^+$ stimulated GnRH release was 29.7±6.0 in controls and 24.8±4.3, 14.5±1.5 and 11.3±0.6 pg/4 min in the 0.1, 10 and 1000 pM OT treated MBHs, respectively. Both inhibition of basal and $K^+$ stimulated GnRH release by OT and the inverse linear correlation between OT dosage and GnRH release were significant at $p<0.01$. Equimolar OT receptor antagonist (ANT; d-$(CH_2)_5$Tyr(Me)AVP) completely prevented the inhibition of basal and $K^+$ stimulated GnRH release by 1 nM OT, whereas ANT alone did not alter GnRH release (n=9/group). When just the ME was incubated with medium alone (n=6) or medium plus 1 nM OT (n=6), OT inhibited both basal (9.5±0.5 vs 30.5±5.4 pg/12 min, $p<0.01$) and $K^+$ stimulated (39.3±5.0 vs 102.1±13.7 pg/4 min, $p<0.01$) GnRH release. These studies demonstrate that excedingly low concentrations of OT can inhibit in vitro GnRH release from male rat MBHs by an OT receptor mediated mechanism, and that this inhibition can be exerted at the level of the ME (i.e. the neurosecretory terminals). These results suggest that endogenous OT may indeed participate in the physiological regulation of GnRH release, perhaps mediating stress or suckling induced inhibition of LH secretion. (Supported by a Mellon Foundation Faculty Scholar Award)

308.10 EFFECTS OF AN OXYTOCIN ANTAGONIST ON THE RESPONSES OF PLASMA PROLACTIN, ADRENOCORTICOTROPIN AND β-ENDORPHIN-LIKE IMMUNOREACTIVITY TO 5-HYDROXYTRYPTOPHAN, SUCKLING AND ETHER STRESS. C. A. Johnston and A. Negro-Vilar. Reproductive Neuroendocrinology Section, Lab. Reprod. Dev. Tox., NIEHS, NIH, Research Triangle Park, N.C. 27709

Recent studies from our laboratory demonstrate the necessity of the neurointermediate lobe of the pituitary (NIL) for the L-5-hydroxytryptophan (5-HTP)-induced increase in plasma prolactin (PRL). Because oxytocin has been shown to exert PRL releasing effects both in vivo and in vitro and is found in nerve terminals within the NIL and median eminence, we analyzed the effect of a potent oxytocin antagonist on plasma PRL concentrations during several experimental paradigms where PRL undergoes a dynamic increase. The oxytocin antagonist [1-deaminopenicillamine, 2-O-methyl tyrosine, 8-ornithine] vasotocin (dPOMeOVT), was graciously supplied by Dr. Maurice Manning (Department of Biochemistry, Medical College of Ohio). The effects of dPOMeOVT (30 µg/kg, i.p., 30 or 40 min prior to sacrifice) on the PRL, adrenocorticotropin (ACTH) and β-endorphin-like immunoreactivity (β-END) responses were examined using the following paradigms: (1) suckling stimulus (30 min by 8 pups following 5-1/2 hrs pup-removal), (2) 5-HTP injection (50 mg/kg, i.p., 15 min) or (3) ether stress stimulus (3 min). Plasma PRL, ACTH and β-END were analyzed by radioimmunoassay. Suckling resulted in a 25-fold increase in plasma PRL and substantial milk ejection as indicated by an average weight gain of 4.4 ± 0.3 grams/group of 8 pups in 30 min. The oxytocin antagonist did not affect basal or suckling-stimulated PRL or basal ACTH and β-END. However, the milk ejection reflex was completely suppressed by the oxytocin antagonist (pups of dPOMeOVT-suckled dams actually lost an average of 2.2 ± 0.9 grams/8 pups). 5-HTP administration caused a large increase in PRL, ACTH and β-END. The oxytocin antagonist did not alter basal or 5-HTP-induced increases in the plasma concentrations of any of these three hormones. Ether stress increased plasma concentrations of all three hormones. Blockade of oxytocin receptors did not statistically significantly affect basal or ether stress-induced changes in PRL, ACTH or β-END. These data suggest that although oxytocin has been reported to demonstrate PRL releasing activity both in vitro and in vivo, this activity is apparently not a major component of the basal secretion of PRL, ACTH or β-END; or of the changes induced in the secretion of those hormones by 5-HTP, suckling or ether stress stimulus. Alternatively, the lack of effect of the oxytocin antagonist on PRL release may suggest that the pituitary receptors for oxytocin are distinct from those present in mammary gland or uterine tissues.

308.11 CHOLECYSTOKIN STIMULATES PITUITARY SECRETION OF OXYTOCIN: EVIDENCE FOR ACTIVATION OF CENTRAL NAUSEA PATHWAYS. J.G. Verbalis, C.M. McHale*, and E.M. Stricker, Departments of Medicine and Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15261.

Previous studies in this laboratory have shown that lithium chloride (LiCl), copper sulfate ($CuSO_4$) and apomorphine, agents assumed to cause nausea in rats because of their effectiveness in promoting the acquisition of learned taste aversions, each stimulated dose-dependent secretion of oxytocin (OT) but not vasopressin (AVP) in male rats. These and other results have suggested that plasma OT might represent a relatively quantifiable marker of visceral illness in rats when other stimuli to OT secretion are absent. Because it has been speculated that cholecystokinin (CCK) may reduce food intake in part due to the production of nausea, we studied OT secretion after administration of synthetic sulfated octapeptide of CCK to rats. Both intravenous and intraperitoneal CCK caused dose-dependent increases in plasma OT, but not plasma AVP, 1-2 minutes after injection (*$p<0.01$ compared to basal):

| CCK(µg/kg) | pOT(µU/ml) | |
|---|---|---|
| | i.v. | i.p. |
| 0 | 2.7 ± 0.4 | 1.3 ± 0.3 |
| 0.1 | 2.7 ± 0.4 | 1.6 ± 0.4 |
| 1.0 | 5.9 ± 0.8* | 3.0 ± 0.3* |
| 10.0 | 11.3 ± 0.8* | 13.8 ± 4.0* |
| 100.0 | 19.3 ± 2.2* | 21.5 ± 4.0* |

Plasma OT levels after CCK administration were comparable to levels after injection of nausea producing agents (LiCl, 1.5 mEq/kg and $CuSO_4$, 2.5 mg/kg), but returned to basal values by 15-20 min after injection. Because OT stimulation occurred with CCK doses near the threshold for its reduction of food intake, further studies were done in rats trained to a twice-daily feeding schedule of liquid diet. In control animals, plasma OT was significantly elevated by 5 min after the onset of feeding and rose to a peak of 10-15 µU/ml after consumption of 17 ± 2 ml in 20 min. These results indicate that pronounced gastric distention associated with rapid food ingestion can also provoke OT secretion, although graded levels of stimulation evidently can occur without sufficient gastric malaise to cause total cessation of eating. Using the same feeding schedule, administration of CCK (10 µg/kg i.p.) 5 min after the onset of eating transiently further elevated plasma OT to 15-20 µU/ml immediately after the injection, and as expected reduced total consumption to only 8 ± 2 ml by 20 min. Conversely, administration of CCK 20 min prior to the feeding period similarly elevated plasma OT levels immediately after the injection, but plasma OT then returned to basal levels by the onset of feeding and subsequent consumption was not reduced. These results are therefore consistent with CCK activation of central nausea pathways to account for at least part of its demonstrated effect to reduce food consumption.

308.12 **NEUROENDOCRINE NEURONAL MAPPING IN THE MACAQUE PROSENCEPHALON BY LUCIFER YELLOW BACKFILLING AFTER INFUNDIBULAR STALK TRANSECTION.** T. Song*, K.K. Thind and P.C. Goldsmith. Dept. of OB/GYN and Repro. Sci., U.C.S.F., San Francisco, CA 94143.

Lucifer Yellow (LY), a non-toxic, highly fluorescent dye, was used as a retrograde tracer to reveal the distribution of the neurons which project to the median eminence in primates. 5% LY in 16% solidified polyvinyl alcohol was applied against the proximal end of the infundibular stalk after high surgical stalk transection in Rhesus monkeys. Two days post-surgery, the animals were perfused with 3% paraformaldehyde/0.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4). The brains were removed, Vibratomed (40 microns) in the frontal plane, and the sections examined under the fluorescence microscope.

Cell bodies with LY-fluorescent inclusions were found in numerous brain regions extending from the nucleus of the diagonal band of Broca (NDBB) back to the medial basal hypothalamus. Perikaryal labeling decreased rostrocaudally in cell bodies localized in: NDBB, and both its vertical and horizontal projections; nucleus accumbens; medial but not the lateral septal nucleus; posterior portion of the medial preoptic area; lateral preoptic area; anterior commissural nucleus; anterior hypothalamic area; dorsomedial and ventromedial hypothalamic nuclei; lateral hypothalamic area; anterior ventral periventricular region; lateral part of paraventricular nucleus; and the dorsolateral region of the supraoptic nucleus. In addition, some LY-fluorescent neurons appeared in thalamic regions, including the terminal striate and paracentral nuclei. Moderately labeled cell bodies were scattered in the globus pallidus. Only a few LY-labeled cell bodies could be observed within the anterior portion of the medial preoptic area and the arcuate nucleus, as has been reported in similar studies in rats (Lechan,R.M., et al., Brain Res., 195:13,1980). The median eminence, and supraoptic, paraventricular, and arcuate nuclei all contained moderately fluorescent fibers.

Some LY-labeled sections were irradiated with intense blue light (490 nm) in diaminobenzidine (DAB) until LY fluorescence disappeared, and then were osmicated and processed for electron microscopy (Maranto,A., Science, 217:953,1982). LY-photooxidized DAB had accumulated in lysosomes more than in perikaryal or fiber cytoplasm. No diffusion of LY-DAB label to adjacent structures was observed.

These results suggest similarities and differences between non-human primates and rodents in their final common neuroendocrine pathways, and offer a means to differentiate (1) preoptico- and tubero-infundibular pathways from preoptico-terminal and possible tubero-preoptic components in the primate; (2) neuropeptidergic vs. monoaminergic contributions to these pathways.

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308.13 DIRECT EFFECTS OF NOREPINEPHRINE, ACETYLCHOLINE AND ESTROGEN ON THE MEMBRANE EXCITABILITY OF HYPOTHALAMIC PARVOCELLULAR NEURONS. T.P. Condon, O.K. Ronnekliev and M.J. Kelly, Department of Physiology, Oregon Health Sciences Univ., Portland, OR 97201

Medial basal hypothalamic (peptidergic) neurons control gonadotropin secretion in the guinea pig. These neurons receive both noradrenergic and cholinergic innervation and are regulated by gonadal steroid feedback. In the present study we have used the in vitro hypothalamic slice preparation from the female guinea pig to examine the effects of norepinephrine (NE) and agonists, acetylcholine (ACH) and estrogen ($E_2$) on the membrane excitability of arcuate (ARC) and cell poor zone (CPZ) neurons, wherein lie the peptidergic (LHRH) neurons controlling gonadotropin secretion. Sagittal hypothalamic slices from cycling (periovulatory) and ovariectomized, $E_2$-primed (OVX-$E_2$) guinea pigs were prepared as previously described (Kelly et al., Brain Res. Bull. 12:399, 1984). Extra- and intracellular recordings were made with procion yellow (PY)- and KCl/KCitrate-filled electrodes. NE ($10^{-7}$ to $10^{-3}$ M), ACH ($10^{-1}$ M), Methoxamine (MEX;α-agonist, $10^{-3}$ M) and $E_2$ ($10^{-8}$ M) were applied via the media or pressure injection. A number of cells were injected with PY and all slices were processed for peptide immunocytochemistry. The PY-identified ARC-CPZ neurons were small (7-15 μm) fusiform neurons, which were found in an area defined immunocytochemically by LHRH and β-endorphin staining. Sixty percent of the ARC-CPZ neurons (n=65) from intact guinea pigs were inhibited by NE. NE inhibition was characterized by membrane hyperpolarization and decreased input resistance ($R_m$). In contrast, 40% of the cells (n=18) recorded from OVX-$E_2$ animals responded to NE with increased firing, which was characterized by membrane depolarization. This excitatory response appears to be mediated by an α-adrenergic mechanism, since MEX application resulted in mostly excitatory responses. In addition several neurons displaying potent NE inhibition were also excited by MEX. Fifty-five percent of the neurons tested with ACH (n=49) showed excitation and only 30% were inhibited. ACH excitation was accompanied by marked membrane depolarization and decreased $R_m$, with $E_2$ priming having no effect on ACH responsiveness. Forty-eight percent of the neurons tested with $E_2$ (n=35) were inhibited with only 9% showing excitation. This $E_2$ inhibition was associated with membrane hyperpolarization and decreased $R_m$ and was independent of $E_2$ priming. Our findings indicate that NE acts via two distinct mechanisms (receptors?) to directly alter the membrane excitability of ARC-CPZ neurons, whereas ACH has a predominant excitatory and $E_2$ an inhibitory role. These findings further elucidate the noradrenergic mechanism(s) involved in the control of gonadotropin secretion. (Supported by PHS Grants HD 19905, HD 16793, and HD 06332.)

308.14 MORPHOLOGICAL EVIDENCE FOR THE EXISTENCE OF NEURONAL AND HUMORAL ULTRA-SHORT FEEDBACK REGULATORY MECHANISMS IN THE HYPOTHALAMUS OF THE RAT. Zs. Liposits, G. Sétáló, V. Csernus and W.K. Paull. (SPON: D.H. York). Depts. of Anatomy, Univ. Missouri-Columbia, Columbia, MO 65212 and Univ. Medical School, Pécs, Hungary.

The production and liberation of diencephalic hypophysiotrophic hormones are hypothesized to be regulated by ultra-short feedback mechanisms. Theoretically, this autoregulatory process might be executed via both neuronal and humoral channels. In order to elucidate the anatomical base of the former mechanism, corticotropin releasing factor immunoreactive (CRF-IR) neurons of hypothalamic paraventricular nucleus were analyzed by light (LM) and electron microscopic (EM)-immunocytochemical methods in long term adrenalectomized rats, which were also treated with dexamethasone (100 μg/100g b.w.) 24 hours before sacrifice. At LM level, CRF-IR axons were seen to contact CRF-synthesizing perikarya. These anatomical arrangements -at EM level- proved to be axo-somatic synaptic connections, indicating that corticoliberin modulates its own production. Local CRF-IR-axons were also observed to innervate magnocellular neurons in the same nucleus. The presence of CRF-IR material in the presynaptic boutons proves the neuromodulator nature of CRF. The possibility of a humoral type ultra-short feedback action of releasing hormones was studied in hypophysectomized female rats, bearing anterior pituitary grafts (obtained from 10 day neonatal rats) in the III ventricle. In one group of recipients one half of the median eminence (ME) was removed prior to implantation. The animals were allowed to survive for 6-12 weeks and consecutive hypothalamic sections containing the graft were immunostained for FSH-β, LH-β, TSH-β, LH-RH and CRF-immunoreactivities at LM and EM levels. Evaluation of the sections showed the following results: 1)Destruction of the ependymal lining of the III ventricle is essential to the formation of a neuro-glandular junction between the graft and the ME. In this case, only fenestrated capillaries (belonging to the subependymal plexus) separated the glandular tissue from neural, allowing humoral communication between them. 2) Pituitary grafts that received blood supply from the subependymal vessels of median eminence-arcuate region contained active, hypertrophied FSH, LH and TSH-immunoreactive cells that exhibited subcellular organelles characteristic for increased hormone production and release, while grafts which had vascularized outside the hypophysiotrophic area (HTA) from vessels lacking connections with the portal system, showed inactive cells. 3)The partial removal of the median eminence resulted in atrophy of pituitary cells on the side of lesion, without affecting the development of hypertrophied cells at the intact side. The morphological and functional maintainance of pituitary grafts, located within the HTA, proves that this territory receives its blood supply from the pituitary portal circulation. Thus it is very probable that neurohormones released into the portal circulation might also effect their own release or production via this vascular route in an ultra-short feedback manner. Supported by NIH grant NS 19266 to WKP.

308.15 irCRF SECRETION OF HYPOTHALAMIC CELL CULTURE: STIMULATION BY NICOTINIC CHOLINERGIC AGONIST. A.T. Lim* and W.Vale. The Clayton Foundation Laboratories for Peptide Biology, The Salk Institute, La Jolla, CA 92037.

Hypothalamic Corticotropin Releasing Factor (CRF) producing cells play the major, but not exclusive, role in the regulation of the secretion of ACTH and other POMC derived peptides by the anterior pituitary. We report here the measurement and regulation of CRF secretion by monolayer cultures of hypothalamic neurons from neonatal rats.

Hypothalami collected from 2-3 day old Sprague-Dawley rats were enzymatically dispersed, washed, counted and resuspended in culture medium; typically the procedure yielded $1{\cdot}5x10^6$ cells per tissue with greater than 95% viability as determined by trypan blue exclusion. $5x10^6$ cells per well were plated on poly-D-lysine pre-coated dishes and cultured in 10% fetal calf serum enriched B-PJ medium at 37°c with 93% air and 7% $CO_2$. Prior to secretion studies, the cells were washed and replenished in Hepes buffered Krebs Ringer bicarbonate solution followed by further 4h incubation with or without various agents. Media were then collected, acidified, lyophylised and reconstituted in assay buffer for rat CRF assay as previously described; routinely, the double antibody radioimmunassay has a sensitivity of 1.5 pg per tube. (Vale, W., et al. Methods In Enzymology., 103:565, 1983).

Basal secretion of CRF by hypothalamic cultures was 3.2 ± 0.2 pg irCRF/dish (mean ± SEM; n=10). High medium potasium as well as acetylcholine and the nicotinic agonist, nicotine, stimulated secretion of irCRF. Nicotine $EC_{50}$ and $E_{max}$ effects were observed at $0.5 \times 10^{-3}$M and $10^{-3}$M releasing 5.8 ± 0.2 pg irCRF/dish and 11.7 ± 0.4 pg irCRF/dish respectively. In the presence of $10^{-4}$M hexamethonium, $10^{-3}$M nicotine released only 4.5 ± 0.3 pg irCRF/dish. $10^{-9}$M of the synthetic glucocorticoid, dexamethasone, actuely (<4 hrs) enhanced the nicotine induced secretion of irCRF; but 24 hour pretreatment with dexamethasone ($10^{-9}$M) strongly inhibited nicotine stimulated irCRF secretion. The mineralocorticoid, deoxycorticosterone was without effect.

We conclude that (i) irCRF is secreted from primary rat hypothalamic cell cultures, (ii) irCRF secretion is stimulated by a nicotinic cholinergic agonist and (iii) the stimulated release of irCRF can be modulated specifically by glucocorticoid.

308.16 PITUITARY CYCLIC AMP AND PLASMA ACTH RESPONSES TO CRF IN VIVO. G.J. Kant, M.A. Oleshansky, D.D. Walczak, E.H. Mougey,* J.L. Meyerhoff. Dept. Med. Neurosciences, Walter Reed Army Institute of Research, Washington DC 20307.

We have previously reported that acute stress elevates levels of pituitary cyclic AMP in vivo. We have found that the stress-induced pituitary cyclic AMP elevations are highly correlated with increased plasma levels of 3 pituitary hormones, ACTH, β-endorphin and β-LPH, thought to be regulated by the hypothalamic peptide, CRF.

The present experiments were performed to determine whether the observed stress-induced increases in pituitary cyclic AMP could be the result of stress-induced CRF release. In all experiments described below, CRF (5 ug in 0.1 ml) was administered via an indwelling jugular catheter fifteen minutes prior to sacrifice by decapitation. Whole pituitaries were rapidly placed in 90°C sodium acetate buffer to inactivate adenylate cyclase and phosphodiesterase. Following sonication and centrifugation, supernatants were stored at -70°C until assayed for cyclic AMP by RIA using antibodies characterized in our laboratory. Trunk blood was collected in the presence of heparin and trasylol (a peptidase inhibitor) and centrifuged. Plasma was stored at -70°C until assayed for ACTH using a commercially available RIA kit.

In the first experiment, CRF administration increased levels of pituitary cyclic AMP and plasma ACTH. Pretreatment with 0.5 ml of CRF antisera 6 min prior to CRF administration greatly attenuated the CRF-stimulated increase in pituitary cyclic AMP seen in animals pretreated with normal rabbit sera.

In a second experiment, 15 min of footshock increased levels of pituitary cyclic AMP in sham-operated but not in pituitary stalk-sectioned rats, indicating that some factor of central origin was required for the stimulation of pituitary cyclic AMP response to stress. Exogenous CRF did increase levels of pituitary cyclic AMP in pituitary stalk-sectioned rats, demonstrating that the pituitaries were still able to respond to appropriate stimuli and suggesting that CRF might be the factor of central origin required.

We have found that the pituitary cyclic AMP response to stress is greatly affected by the time of day of stress exposure. Fifteen min of restraint stress at "lights on " (0600) increased levels of pituitary cyclic AMP over 10 fold while the same stressor failed to elevate cyclic AMP levels at "lights off" (1800). The plasma ACTH response to stress was also much greater at 0600. Therefore, in a third experiment, we administered exogenous CRF as above to rats at "lights on" and at "lights off". CRF adminstration at light onset markedly increased levels of pituitary cyclic AMP and plasma ACTH; while CRF given at the beginning of darkness had no effect on either cyclic AMP or ACTH levels. These findings may be related to the reported diurnal rhythm in endogenous CRF release.

Taken as a whole, these data support the hypothesis that the primary factor responsible for observed increases in levels of pituitary cyclic AMP following acute stress is CRF.

308.17 EFFECTS OF EPINEPHRINE ON PITUITARY CYCLIC AMP AND PLASMA HORMONES IN VIVO. E.H. Mougey*, G.J. Kant, D.R. Collins*, L.L. Pennington*, J.L. Meyerhoff. (SPON: C.F. Tyner) Dept. Med Neurosciences, Walter Reed Army Institute of Research, Washington D.C. 20307.

Although CRF appears to be the primary stimulus for ACTH release from the pituitary, both in vitro and in vivo studies suggest that catecholamines may also be important regulators of ACTH release. Epinephrine markedly potentiates CRF-stimulated ACTH release from pituitary preparations. In vivo, a combination pretreatment of CRF antisera and chlorisondamine, a blocker of peripheral catecholamine release, attenuates stress-induced ACTH release more effectively than either pretreatment alone.

We have reported that various stressors elevate levels of pituitary cyclic AMP. The stress-induced cyclic AMP response is highly correlated with the release of pituitary hormones thought to be regulated by CRF (ACTH, β-endorphin, β-LPH). Since epinephrine has been reported to potentiate CRF-stimulated pituitary cyclic AMP synthesis and since stress elevates levels of plasma epinephrine, the present study was conducted to characterize the effects of epinephrine on levels of pituitary cyclic AMP and plasma hormones in vivo.

Rats were injected with saline or epinephrine bitartrate (1 mg/kg IP) and sacrificed by decapitation 1, 5, 15, 30 or 60 min post-injection. Rats remained in their home cage until sacrificed. In addition, one group of rats was sacrificed without injection (controls). Whole pituitaries were placed in 90°C sodium acetate buffer for 15 min to inactivate adenylate cyclase. Pituitaries were then sonicated and centrifuged. Supernatants were stored at -70°C until assayed for cyclic AMP by RIA. Trunk blood was collected with heparin/aprotinin and centrifuged at 4°C. Plasma was stored at -70°C until assayed for adrenocorticotropin (ACTH), β-endorphin (β-EP), β-lipotropin (β-LPH), corticosterone (CS) and prolactin (PRL) by RIA.

Saline injection did not increase levels of pituitary cyclic AMP at any of the time periods at which animals were sacrificed. ACTH levels were somewhat elevated 5 min after saline injection but returned to control levels by 30 min. Corticosterone levels were increased at 15 min and remained elevated at 30 and 60 min. Prolactin, β-EP and β-LPH levels were not significantly increased by saline injection.

Epinephrine injection resulted in 4 fold increases in pituitary cyclic AMP levels at 15 min and 14 fold increases by 30 minutes. ACTH, β-EP and β-LPH levels were significantly elevated at 15, 30 and 60 min and CS levels were significantly elevated at 30 and 60 min compared to saline injected animals. Epinephrine injection, however, did not elevate PRL levels.

These data demonstrate that epinephrine can stimulate pituitary cyclic AMP synthesis and pituitary hormone release. Increases in plasma levels of the three hormones derived from the common precurser, proopiomelanocortin, are highly correlated whereas plasma PRL appears to be unaffected by epinephrine stimulation.

308.18 PLASMA CORTICOSTERONE RESPONSES TO ELECTRICAL STIMULATION OF THE BED NUCLEUS OF THE STRIA TERMINALIS. Jon D. Dunn, Dept. Anat., Sch. Med., Oral Roberts Univ., Tulsa, OK 74171.

Previous studies reported from this laboratory have shown that electrical stimulation of cytoarchitecturally distinctive sites within the amygdala results in differential plasma corticosterone responses. To further pursue the question of differential limbic influences on pituitary-adrenal function plasma levels of corticosterone (Cpd B) obtained prior to and following sham or electrical stimulation of the bed nucleus of the stria terminalis (BNST) were determined for adult female rats which had been anesthetized with urethane (1.3 g/kg). All rats were tracheotomized, placed on a heating pad and subsequently positioned in a stereotaxic apparatus. Hippocampal EEG, ECG, heart rate, blood pressure and respiration were routinely monitored; timed blood samples (0.2ml) were obtained from a catherized tail artery. Samples were taken at 0.5 min. prior to and at 5, 10, 15 and 30 min. after initiation of stimulation (monophasic square waves, 100μA, 50HZ, 0.5 msec, 1 sec on/1 sec off for 30 min.). For purposes of plotting corticosterone responses to stimulation a change in plasma Cpd B was considered different from no change when the average of the 5, 10 15 and 30 min. samples deviated by more than 10% from the pre-stimulus level. However, statistics were derived from Cpd B responses grouped according to stimulation site.

Whereas increased ($P<0.05$) plasma Cpd B levels followed stimulation of the medial aspect of the BNST, stimulation of the lateral aspect of the BNST resulted in decreased ($P<0.05$) plasma Cpd B levels. The overall increase in plasma Cpd B following medial stimulation was 14%; the overall decrease was 19%. The largest increase in plasma Cpd B (32%) occurred at 30 min. post-stimulation; the largest decrease (31%) occurred at 15 min. Stimulation of the most rostral or pericommissural aspect of the BNST produced plasma corticosterone responses similar to that observed following medial stimulation. In contrast no change in Cpd B levels were observed following either sham stimulation or stimulation of the corpus callosum, fornix or anterior commissure.

Considering the known amydaloid-septal connections, these data are consistent with our previous findings in that the evoked Cpd B responses paralleled those evoked by amygdaloid stimulation. These data also indicate that differential plasma Cpd B responses can be evoked from limbic forebrain areas other than those of the amygdala and hippocampus.

308.19 HALOPERIDOL INCREASES PLASMA BETA ENDORPHIN AND SERUM CORTISOL IN NORMAL HUMAN MALES. MM Murburg,* D Paly,* RC Veith,* KL Malas* and DM Dorsa (SPON: W Stahl) Departments of Psychiatry and Behavioral Sciences, Medicine, and Pharmacology, University of Washington, and VA Medical Center, Seattle WA 98108.

Dopamine plays a role in regulating pituitary secretion of certain pro-opiomelanocortin derived peptides, such as beta endorphin (βE) and adrenocorticotropin (ACTH) in animals. In rats, for example, the dopamine-blocking neuroleptic haloperidol causes an acute increase in plasma βE and ACTH (Giraud et al: Eur J Pharmacol 62:215-217, 1980), and dopamine inhibits release of βE and gamma endorphin from neurointermediate lobe (Dorsa et al: Endocoids, H Lal, AR Liss, Inc, 1984). To invesigate possible dopaminergic regulation of pituitary function in man, we have tested the effects of a single intravenous (IV) dose of haloperidol on plasma levels of βE and serum levels of cortisol in normal human subjects.

Ten drug and alcohol free normal males, aged 24 to 38, giving informed consent, were randomly assigned (five each) to treatment with intravenous haloperidol 0.03 mg/kg or placebo (sterile saline), which were administered between 10 and 11 in the morning following a 10-hour fast and abstention from caffeine and cigarettes. Arterialized venous blood samples were collected over a 30-minute period prior to injection (three samples) and at 5 to 30-minute intervals following injection (14 samples). Samples were extracted on a silica column (βE) and analyzed by radioimmunoassay for βE and cortisol.

Baseline βE and cortisol values were calculated using the first three (pretreatment) values for each individual. There was a significantly greater % increase from basal values of βE ($F = 6.62$; $df = 1.16$; $p \leq .05$) and cortisol ($F = 43.6$; $df = 1.16$; $p \leq .01$) in the haloperidol-treated, compared to the placebo-treated, subjects by two way analysis of variance. The mean increase in βE concentration for the haloperidol-treated subjects over the 210 minutes studied was significantly greater at 12.8 pg/ml ($p \leq .05$, Wilcoxon one-tailed t test) than the mean increase in βE concentration for placebo-treated subjects, 4.8 pg/ml. The mean change in cortisol concentration for haloperidol- treated subjects was 3.6 mcg/dl, significantly greater than the mean change for the placebo-treated subjects, -4.4 mcg/dl ($p \leq .05$, Wilcoxon one-tailed t test).

This data indicates that administration of intravenous haloperidol to normal human males causes acute release of βE and cortisol into plasma, compatible with dopaminergic regulation of human pituitary βE and ACTH secretion.

308.20 NALOXONE EFFECTS ON PLASMA [VASOPRESSIN] AND [OXYTOCIN] ELEVATED BY HISTAMINE, NICOTINE AND INCREASED [NaCl] IN CSF. J. Summy-Long, C. Denlinger*, D. Palm*, R. Hartman* and L. Rosella-Dampman*. Dept. Pharmacol., M.S. Hershey Med. Ctr., Penn. State Univ., Hershey PA, 17033.

Endogenous opioid peptides (EOP) inhibit release of oxytocin (OT) during dehydration and hemorrhage and thereby allow the preferential release of vasopressin (VP) from the hypothalamo-neurohypophysial system when it is of physiological importance (Brain Res. 309:362, 1984). EOP also attenuate release of OT during parturition (J. Steroid Biochem 20:1502, 1984) and secretion of VP by tail electroshock (J. Pharmacol. Exp. Ther. 226:373, 1983). It has been proposed from in vitro studies that EOP inhibit release of neurohypophysial hormones by an action on the neural lobe. We therefore investigated the effect of an opiate receptor antagonist, naloxone (NAL), on the plasma concentrations of OT and VP elevated by various pharmacologic stimuli, including histamine (HIS), nicotine (NIC) and increased [NaCl] in cerebrospinal fluid (CSF). If OT (or VP) release were attenuated by EOP regardless of the stimulus, the site of opiate action may be in the final common pathway, i.e. the neural lobe or magnocellular neuron. Adult male Sprague Dawley rats were injected s.c. with saline (Sal; 1 ml/kg) or NAL (5 mg/kg) 5 min before HIS (10 mg/kg i.p.), NIC (0.15 mg/kg or 1.5 mg/kg i.p.) or saline (control; 1 ml/kg i.p.). Rats with a cannula in the lateral cerebroventricle were injected intracerebroventricularly (IVT) with 10 μl of artificial CSF (0.16 M NaCl) or CSF containing 1 M NaCl. Animals were decapitated 60 sec (↑ [NaCl] CSF) or 10 min (HIS and NIC) after the stimulus or vehicle. VP and OT were extracted from plasma and quantified by RIA. Differences among groups (n5-15 rats/group) were determined by AOV, Newman Keuls and Student's t tests. The concentrations (pg/ml; x ± SE) of OT and VP in plasma were elevated ($p < 0.05$) by HIS (OT 10 ± 1 vs 36 ± 5; VP 1 ± 0.3 vs 91 ± 13), ↑[NaCl] CSF (OT 19 ± 2 vs 226 ± 77; VP 3 ± 0.1 vs 71 ± 22) and NIC at the high (OT 7 ± 0.5 vs 58 ± 7; VP 3 ± 0.3 vs 38 ± 7) but not low (OT 8 ± 1; VP 2 ± 0.1) dose. NAL increased further ($p < 0.05$) [OT] in plasma after HIS (224 ± 38), NIC (0.15 mg/kg, 25 ± 5; 1.5 mg/kg, 317 ± 49) and ↑[NaCl] CSF (880 ± 207). NAL also increased ($p < 0.05$) [OT] in controls (NAL-CSF 38 ± 6, NAL-SAL 15 ± 2). [VP] was unaffected by NAL in either controls (NAL-CSF 4 ± 0.4; NAL-SAL 3 ± 0.3) or stimulated (NAL-HIS 121 ± 30; NAL-NIC, 0.15 mg/kg 2 ± 0.3, 1.5 mg/kg 40 ± 8.0; NAL-↑[NaCl] CSF 82 ± 9) rats. NAL increased plasma [OT] but not [VP] in response to each stimulus. The data are consistent with the hypothesis that endogenous opioid peptides inhibit release of OT in vivo by an effect on the final common pathway, i.e. the magnocellular neuron or neural lobe (supported by NIH HL32826).

309.1 NEUROMUSCULAR PLASTICITY: A CRITICAL PHASE. C. Winkelmann, B.T. Stokes, P. Gorman*, P.J. Walters*, and P.J. Reiser*. Dept. of Physiology, Ohio State Univ., Sch. of Med., Columbus, OH 43210.

Previous work in our laboratory and elsewhere suggests that a critical developmental phase exists for the final stage of differentiation of the latissimus dorsi muscle group in the chick embryo. It is the hypothesis of our group that this final development of fast twitch muscle characteristics is directly under the influence and is precipitated by alterations in motor unit activity that occur during this period. To test this hypothesis, we have conducted a series of studies on the electromyography of the PLD (posterior latissimus dorsi-fast twitch) and ALD (anterior latissimus dorsi-slow twitch) muscles in situ from 13-20 days of development. In addition, we have studied the normal and altered development (neuromuscular blockade) of the mechanical characteristics of the PLD in vitro during this same time period. Such a regimen has allowed us to distinguish those characteristics of myogenesis that are most dependant on functional innervation during development.

The electromyographic studies revealed that clear differences exist in the spontaneous output of motor units to the ALD and PLD during this period. While burst frequencies and content are essentially the same to the ALD and PLD, a considerable decrease in the interburst interval takes place only to the PLD fibers. During this period, the PLD is also undergoing inordinate changes in its functional characteristics. Normalized peak tension, time to peak tension, maximal rate of force development, and time to half relaxation, all essentially achieve their adult characteristics by changing 334%, 66%, 354% and 437%, respectively, from 14-20 days in ovo. These data provide a temporal framework for the investigation of the effects of functional immobilization on the development of this model neuromuscular system.

Isometric contractile properties of PLD muscles isolated from control (saline injected) and chronically immobilized (days 8-18 in ovo) embryos were compared at 18-19 days in ovo. Embryonic motility observations indicated that the immobilization protocol resulted in a motility reduction of >80% in treated embryos from days 10-18 in ovo. Compared to control embryos, mass, length and cross-sectional area were considerably reduced in immobilized animals. In addition, normalized twitch and tetanic tensions were considerably reduced while the relaxation phase of the response became much longer. The time to peak tension for the twitch response doubled while that for the tetanus did not change. These alterations suggest that the functional activity of the sarcoplasmic reticulum (as revealed in the relaxation kinetics) is much more dependant on activity in developing muscle than is the myosin enzyme composition (rising phase kinetics). (Supported by the Muscular Dystrophy Association)

309.2 **DEVELOPMENTAL EXPRESSION OF TRANSMITTER PHENOTYPE IN DOPAMINERGIC NEURONS.** E.G. Sieloff, M. Lazoff, J. McLean and M.T. Shipley. (SPON. G. Blaha). (University of Cincinnati College of Medicine).

The laminar architecture of the main olfactory bulb (MOB) makes it a useful model for studying the factors that regulate the development of neuronal specificity. To investigate the relationship between neuronal development and neurotransmitter expression in the MOB, we have studied the expression of the enzyme tyrosine hydroxylase (T-OH) in dopaminergic (DA) neurons.

In the adult rat, DA is found only in superficial tufted and periglomerular neurons. These cells are born in the ependymal zone and migrate to their destinations in the most superficial layer of MOB (Hinds, 1968). Three possibilities exist for the developmental expression of the DA phenotype. The enzyme may be expressed: 1) at neuronal birth, 2) as the neurons are migrating from the proliferative zone, or 3) after the neurons are inserted into their definitive circuit matrix.

The expression of T-OH in MOB of developing rats was demonstrated by immunocytochemistry. We found that T-OH activity is expressed only after neurons have migrated to the glomerular and superficial part of the external plexiform layers. The progressive increase in DA neuron cell number parallels the ingrowth and maturation of primary afferent and centrifugal fiber systems that terminate in the glomeruli. By ca. 37 days most DA neurons appear to have reached the periglomerulur region but they are crowded in the deeper parts of the glomeruli. Over the next 20-40 days, the neurons redistribute themselves and encapsulate the glomeruli as in the mature bulb.

Removal of primary olfactory neuronal (PON) input to the MOB causes a transient loss of DA activity in the adult bulb, (Kawano and Margolis, 1982) suggesting that the maintenance of the DA phenotype depends upon PON inputs. The present results suggest that the developmental expression of the DA phenotype may also be modulated by synaptic contacts. Alternatively, the signal for T-OH expression may be the formation of contacts between DA neurons and their target cells in the glomeruli.

Given the ordered and relatively simple structure of the glomeruli and our growing knowledge of the ontogenetic development of their circuitry, it should be possible to identify the factors regulating T-OH expression for these central DA neurons. Such information may improve our understanding of the events influencing the survival and function of dopamine neurons implanted to alleviate Parkinson's disease.
Supported by: NIH NS 19730, NINCDS 18490; US ARMY DAMD-82-C-2272 and DOD DAA G-83-G0064.

309.3 COMBINED LINEAGE AND DYE-COUPLING STUDIES OF NEUROGENESIS IN XENOPUS LAEVIS. C.F. Ide, J.S. Morrow*, R.W. Snow, J.S. Ahluwalia*, and F.E. Dudek. Dept. of Biol., Tulane Univ., New Orleans, LA 70118 and Dept. of Physiol., Tulane Univ. Sch. Med., New Orleans, LA 70112.

Although gap junctions are generally thought to be important in development, their possible role in determination of neuronal phenotype remains unclear. To define the relative contributions of cell lineage and gap-junctional communication in setting neural cell fate, we simultaneously labeled embryonic cells with a lineage marker (horseradish peroxidase, HRP) and a dye that permeates gap junctions (Lucifer Yellow, LY) to determine the subsequent identity of the dye-coupled neuronal phenotypes.

LY and HRP (4% wt/vol) were simultaneously pressure-injected from micropipettes into single cells of embryos in the midblastula (6-7) stage. The extent of dye injection was determined by briefly viewing LY fluorescence with a silicon intensified target camera. Animals were allowed to develop to embryonic stage 38-40, fixed for 10 min in 1% phosphate-buffered (pH 7.3) paraformaldehyde, and reacted for HRP using diaminobenzidine. Developed embryos were post-fixed for 20 min in 3% paraformaldehyde, embedded in plastic (Immuno-bed) and sectioned at 8 μm. We assayed which phenotypes and/or regions were marked by HRP-LY and which were marked with LY alone. Labeled cells appeared in adhesive gland, olfactory epithelium, retina, lens, prosencephalon, mesencephalon, and rhombencephalon. When HRP-LY was found in mesencephalon, rhombencephalon and/or retina, LY appeared alone in prosencephalon; in other embryos, the opposite was true. A similar relationship was seen between olfactory epithelium and lens.

Since LY appears in phenotypes and regions other than those marked by HRP-LY, cells which are dye-coupled during early development do not necessarily share the same range of potential cell fates. Thus, cells may be interacting via junctional communication at the time they (and their potential progeny) become channeled into different determination pathways. Injection at progressively later developmental stages should reveal if other (and more restricted) specific HRP-LY:LY combinations appear as development proceeds.

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309.4 THE AMPHIBIAN MAUTHNER CELL IS DETERMINED DURING VERY EARLY NEURULATION. D.H. Schlenoff* and P.G. Model. Dept.Neurosci., Albert Einstein Coll. Med., Bronx,N.Y. 10461.

The Mauthner cell (M-cell) is a uniquely identifiable neuron found as a single pair in the medullae of fish and premetamorphic amphibians. In the axolotl (Ambystoma mexicanum), they are first recognizable in late tailbud embryos. The cells that give rise to M-cells undergo their final DNA synthesis long before this time, during late gastrulation, just prior to the onset of neurulation. Experiments were conducted to establish the time at which the M-cell is determined, i.e., when it becomes restricted or irrevocably committed to its particular developmental pathway.

Using a classical approach, small pieces of prospective medulla containing the prospective M-cell were unilaterally excised from embryos at various stages of development and transplanted to the flank of host embryos. Light microscopic examination of young larvae revealed that grafts from embryos in which the neural folds had just formed around the neural plate (second half of stage 14) and older embryos contained M-cells, whereas the operated sides of donor medullae did not. Grafts contained both gray and white matter and often formed a ventricle-like area devoid of cells. Many of the grafts from mid-neurula stages contained two M-cells, perhaps indicating that the graft had been removed from inhibitory influences acting on one of the sister cells after the final cell division of the M-cell precursor.

Operations were similarly performed on very early neurulae (first half of stage 14). Grafts from these embryos sometimes contained M-cells and sometimes did not. In those experiments in which grafts did not contain these cells, the medullae of the donors were fully regulated and contained normal pairs. Later, by the second half of stage 14, the M-cell is present in grafts but not in donors. Clearly, determination of the M-cell occurs in the very early neurula. In one instance in which an embryo was operated on in the middle of stage 14, both the graft and the donor contained M-cells: this indicates that the period of determination continued for a short time after the cell in the graft had been committed to the M-cell pathway thereby allowing complete regulation of the donor.

We have established that the fate of the M-cell precursor is specified during very early neurulation, before the determination of many other neurons of the CNS.
(Supported by NIH grant NS-18823).

309.5 DEVELOPMENT OF SUBSTANCE P IMMUNOACTIVITY IN XENOPUS EMBRYOS. B.C. Gallagher* and S.A. Moody. Department of Anatomy, University of Virginia, Charlottesville, Virginia 22908.

A recent report (Clarke, J.D.W. et al, J. Physiol. (1984), 348, 511-525), indicates that HRP-labeled antibodies to substance P stain Rohon-Beard cells in whole mounts of stages 37/38 Xenopus embryos. Developmental processes which lead to differentiation can be followed using an early-differentiating cell such as the Rohon-Beard neuron, and the production of a phenotypic peptide would aid the identification of these cells. The present study characterizes substance P-like immunoactivity when performed on sections of Xenopus embryos, in order to determine whether the staining is confined to Rohon-Beard neurons using this method and at what stages substance P-like immunoactivity is detected.

In the adult spinal cord of Xenopus, the only immunoactive cell bodies are those of the dorsal root ganglia. At stages 37/38, substance P-like immunoactivity is seen in ventral diencephalic neurons. Trigeminal ganglion cells are stained, as well as peripheral axons of that nerve to the cement gland and central axons in the ventro-lateral tract. In the rhombencephalon, cranial interneurons and large basal plate neurons show substance P-like immunoactivity. Stained axons are present in the cranial myotome with apparent synaptic varicosities. In the spinal cord, Rohon-Beard cells are consistently stained. Ventral neurons no longer stain as frequently, but interneurons stain in this region as well as the cranial region. Substance P-positive axons in the dorsal myotome compartment exhibit apparent synaptic varicosities near myotubes.

At stages 29/30, fewer cells stain but the pattern of immunoactivity is similar to that seen at stages 37/38. At stage 21, the time of axonal outgrowth of many primary neurons (Huang and Jacobson, in press), substance P immunoactivity could not be detected by the method employed in this study.

Using standard immunohistochemical procedures on frozen sections of Xenopus embryos, we have demonstrated that several neurons other than Rohon-Beard cells produce substance P. These cells all appear to be among those first born (primary neurons) in the frog CNS, as described by Lamborghini, J.E., J. Comp. Neurol. (1980) 189, 323-333. These cells are not immunoactive at developmental stages at which axonal outgrowth is beginning, but are strongly immunoactive at stages in which target cells have been contacted. Furthermore, immunoactive axons with several varicosities were found not only in the typical "sensory" areas (i.e., in the cement gland and under the epidermis), but also among muscle cells. Supported by NIH NS20604.

309.6 **RETINAL NEUROGENESIS IN EMBRYONIC AFRICAN CICHLID FISH** **Linda C. Shelton*, Russell D. Fernald and Joelle C. Presson, Institute of Neuroscience, Univ. Or., Eugene, Or. 97403**

**Teleost fish grow throughout life and their eyes enlarge correspondingly. Retinal area increases as a result of new retinal cells of all types being added concentrically from the retinal blastemal zone around the iris margin. In addition, newly generated rods are intercalated into existing retinal tissue which is being stretched. The production of rods, after the generation and differentiation of all other retinal cell types, may recapitulate the generation of retinal cells in the embryo, an hypothesis we wished to test.**

**We have analyzed embryonic retinal neurogenesis in the mouthbrooding African cichlid fish, Haplochromis burtoni. Time of fertilization was obtained by behavioral observation of spawning, and mothers with fertilized eggs kept in isolated tanks. Normally, the eggs hatch into larvae in the mothers mouth, and free swimming fry emerge in 12-14 days. By this time the retina is adult-like in structure and growth pattern. At various times during development, larvae were removed from the mothers' mouth and injected with $^3$H-thymidine. The animals were allowed to survive for 30 minutes to 1 month before they were sacrificed, fixed, embedded in plastic, sectioned at 3-5μ and processed for autoradiography.**

**Embryonic teleost retinal development proceeds along a vitread-sclerad gradient similar to that found in other vertebrates. Differentiation begins just dorsal to the location of the optic nerve in the developing eye cup. Four days after fertilization, the beginning of organization within the (inner plexiform layer) IPL is seen and ganglion cells are located near the vitreal surface. By four and a half days, (inner nuclear layer) INL organization of differentiated cells is evident and at five days, cells in the (outer nuclear layer) ONL are distinct but unidentifiable at the light level. Subsequently, a second developmental gradient occurs along the ventro-temporal to dorso-nasal axis. This appears as a wave of cell division, seen only in the ONL. From $^3$H-thymidine labelling, we know that these cells arise from a source which can be labelled at day 4 and which is in the temporal region of the developing eye. The cells produced appear to be rod nuclei at the light level.**
**Supported in part by NIH EY 05051 to RDF.**

309.7 EMBRYONIC DEVELOPMENT OF THE SONIC MOTOR NUCLEUS IN THE OYSTER TOADFISH, OPSANUS TAU. A.J. Galeo, M.L. Fine and J.A. Stevenson. Depts. of Biology and Anatomy, Virginia Commonwealth University, Richmond, VA 23284.

The sonic motor nucleus (SMN), a likely homoloque of the hypoglossal nucleus, provides the final common pathway for sound production in the oyster toadfish (Opsanus tau). Previous work indicates that SMN neurons increase in size and number for 7 to 8 years postnatally. This study attempts to describe normal embryonic development of the SMN and to experimentally demonstrate cell origin, migratory route and rate of addition of neurons pre- and postnatally using 3H-thymidine autoradiography. Toadfish embryos were reared in the lab, fixed daily and staged following Tracy (1959). The sonic apparatus differentiates late in development. The swimbladder develops (Tracy stage 5, myotome motility) as an outpoocketing of the gut, and the sonic muscle from myotome 2 and its encased sonic nerve grow down from the occipital spinal cord (stage 7, attached larva) and adhere to the swimbladder wall (stage 8, free swimming).

An SMN containing between 27 and 50 cells was first recognizable at 19 days post fertilization. Neuron number increases slightly through stage 6 and more dramatically in stages 7 and 8, with stage 8 averaging about 330 cells. Mean soma area increases gradually between stage 5 (21$\mu m^2$) and 6, levels off at about 30 $\mu m^2$ (early and late 6 and 7), and increases at stage 8 (51$\mu m^2$). Mean nuclear area follows a similar pattern. SMN volume increases from about 2.3 X $10^3$ $\mu m^3$ in stage 5 to 124 X $10^3$ in stage 8. Initially, the SMN is situated entirely within the spinal cord, but it grows forward during development so that by stage 8 the rostral pole extends into the medulla, just beneath the fourth ventricle. This finding provides further support for our suggestion of hypoglossal homology.

3H-thymidine autoradiography demonstrates that SMN cells are added slowly but continuously during embryonic development. Labeled cells are also present in young and young adult (sexually mature) toadfish, in agreement with our previous finding of long-term ontogenetic changes in the SMN. Cells originate in the ventral ependymal lining of the central canal and migrate downward to assume their final position in the nucleus.

Supported by a grant from NIMH.

309.8 MIGRATION BY PERIKARYAL TRANSLOCATION FROM THE RHOMBIC LIP IN THE CHICK EMBRYO MEDULLA. K. Book*, J. Ganchrow*, and D.K.Morest. Anatomy Dept., U. Conn. Health Center, Farmington, CT 06032.

The matrix zone bordering the rhombic lip is the source of a large number of nuclear groups in the medulla. Golgi, reduced silver, and toluidine blue methods have been used in Hamburger-Hamilton staged embryos (E4-E8) to trace the migration routes to the auditory and vestibular nuclei from the rhombic lip between cranial nerves V and IX. The neuroblasts migrate by elongation and perikaryal translocation. Three main migratory pathways have been identified in this region: medial, ventral, and circumferential. Particular groups of neuroblasts favor specific migration routes depending on their destination and neuronal type. These migration routes have no consistent relationship with radial glial cells (or primitive epithelial cells).

The migration of neuroblasts to nucleus magnocellularis (avian homologue of the cochlear nucleus) provides one example of migration and early differentiation of neuroblasts in this area and has been studied in detail. Prior to migration (E4.5-5) the neuroblasts have their perikarya in the matrix zone and possess a primitive ventral process, which may bend laterally and remain within the rhombic lip or penetrate the mantle zone. This process grows out of the matrix zone, often led by a large foliate growth cone. At this time cochlear nerve fibers, tipped with growth cones, approach and may even enter the matrix zone in this region. By E6 the anlage of nucleus magnocellularis can be located in the mantle layer beneath the rhombic lip. The perikarya of neurons migrating to this nucleus may still remain in the matrix zone while extending a medially directed axon from the ventral process. The axon may bifurcate into medial and lateral branches in the presumptive nucleus or in the transition area between the matrix and mantle zones. This transition area provides a region where the leading and trailing processes of the neuroblasts tend to spread out horizontally. Migration is consummated by a translocation of the perikaryon to the vicinity of the axon's origin. The post-migratory cell becomes oriented either perpendicular to the matrix zone (rostrally) or diagonal to it (caudally); at this stage it sends an axon into the crossed tract, typically with a collateral recurving dorsally. The young post-migratory neurons have few dendrites and are fusiform in shape. At E7-E8 dendrites and axonal branches begin to spread out. (Supported by NIH grant RO1 NS14354.)

309.9 DIFFERENTIATIVE CAPACITY OF AVIAN NEURAL CREST CELLS INTO SKELETAL MUSCLE CELLS IN THE EYE MUSCLE. T. Yamashita* and G.S. Sohal (SPON: T.M. Nosek). *Dept. of Anat., Sch. of Med., Kanazawa Univ., Kanazawa 920, Japan; Dept. of Anat., Med. Coll. of Georgia, Augusta, GA 30912.

It is known that cranial neural crest cells differentiate into neural, skeletogenic, connective, and glandular tissue cells. However, concerning skeletal muscle cells, their differentiative capacity remains obscure.

The cranial neural crest together with the adjacent neural fold of duck embryos at stage 9 of Hamburger and Hamilton was removed or replaced with that of quail embryos at the same stage. The iris of the operated embryos on day 20 was examined by light and electron microscopy and compared with that of normal duck and quail at the comparable stage. Quantitative analysis of nuclear heterochromatin of the skeletal muscle cells in the iris of duck, quail and their chimaera was performed by cutting out the nucleus profiles from electron micrographs, cutting out the heterochromatin profiles from the isolated nucleus profiles, and then weighing them.

Extirpation of the cranial neural crest resulted in formation of small irides containing smooth muscle cells but lacking skeletal muscle cells. Transplantation of the cranial neural crest from quail to duck produced normally-organized irides containing numerous cells which showed a large heterochromatic mass peculiar to quail cells. However, distinction of skeletal muscle cells from the other kinds of cells in the iris, such as smooth muscle cells, mesenchymal cells, and Schwann cells, was difficult under light microscope.

Quantitative analysis of nuclear heterochromatin revealed that 23 (8%) among 283 nucleus profiles of skeletal muscle cells in the chimaera iris showed an enormously large heterochromatic mass of 2.3 $\mu m^2$ or more in area, which was found in 16% of those in the quail iris but in none of those in the duck iris. The proportional value of average heterochromatin size per nucleus profile to average nucleus size was 9% for the chimaera. This value corresponds to that (10%) for the quail but not to that (3%) for the duck.

Based on these findings, it is concluded that avian neural crest cells can differentiate into skeletal muscle cells.

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309.10 MONOCLONAL ANTIBODIES THAT DISTINGUISH AMONG NEURONAL SUBSETS IN THE SPINAL CORD OF THE CHICK EMBRYO. M.J. O'Donovan[1], R.T. Caldwell*, J.A. Morrison*, and J.L. Denburg. Depts. of Biology and Physiology & Biophysics[1], Univ. of Iowa, Iowa City, IA 52242.

The nervous system contains many different subsets of neurons. Hybridoma techniques have been used to isolate monoclonal antibodies (MAbs) that selectively bind to and thus identify some of these neuronal subsets. We have applied these techniques to help in the identification of subsets of neurons in the dorsal root ganglion (DRG) and spinal cord of the chick embryo.

Spinal cords were removed from 7 day embryos, sonicated in phosphate buffered saline and centrifuged at 100,000 g for one hour. Each mouse was injected with the insoluble pellets from 7 chicks. Spleens were isolated and fusions were performed in the usual manner. Hybridoma supernatants were screened for binding to frozen sections of stage 28 embryos in the region of the lumbosacral cord. The embryos had been fixed for 2 hours in 4% paraformaldehyde.

In the 7 day chick embryo motor and DRG neurons can be identified morphologically and physiologically. Although less is known about spinal interneurons, those of the hindlimb pattern generator are already functional. Therefore, neuronal subsets of the chick nervous system are already present at this stage of development. We have been able to isolate MAbs that selectively bind to some of these subsets.

As expected most of the MAbs bound to some extent to all the cells in the embryo. A small number bound selectively to the nervous system and the adjacent myotome. However, of particular interest were those that selectively labeled some cells within the nervous system. Three categories of such MAbs have been isolated. These include:

1. Those that bind only to CNS neurons
2. Those that bind only to peripheral neurons
3. Those that selectively bind to neurons in the lateral motor column and the DRG.

These results indicate that there are molecular differences between central and peripheral neurons at this stage of development. The developmental regulation and molecular characterization of these molecules is currently under investigation. In addition, the molecular similarities between motor and DRG neurons could be the result of the early maturation of these cell types or related to their pattern of peripheral and central synaptic connectivity.

(Funded by NIH grant NS 14295)

309.11 A DEVELOPMENTALLY REGULATED GANGLIOSIDE IN THE RAT CENTRAL NERVOUS SYSTEM DEFINED BY A MONOCLONAL ANTIBODY. A. S. Blum*, M. Constantine-Paton and C. J. Barnstable. Lab. of Neurobiology, The Rockefeller Univ. New York, N.Y. 10021.

A monoclonal antibody, JONES, displays discrete binding in the developing rat CNS. Punctate labelling is seen in the ventral forebrain, the optic vesicle, and the rhombic lip of the hindbrain by E9. Later, by PN1, JONES binding reveals a dorsal to ventral gradient distribution across the retina. From PN7, JONES binding decreases in the retina and in the adult, persists only as a punctate band in the outer plexiform layer.

We observed that JONES immunoreactivity in the retina was labile to organic solvent pretreatment of cryostat sections. These data suggested that JONES might recognize a lipid. To test this, postnatal rat retinas were extracted using a modification of the Folch method (Folch,J. et.al., J. Biol. Chem., 226: 497, 1957). The extracted whole lipid fraction was subjected to thin layer chromatography (TLC). We adapted the method of Towbin, (Towbin,H. et.al., J. Imm. Meth., 72: 471, 1984), to blot the separated lipids onto nitrocellulose. They were then reacted with JONES and visualized with a HRP-conjugated second antibody. A single immunoreactive band was found. The complex lipid fraction was then extracted with 0.1M aqueous KCl which separates gangliosides from most other contaminating lipids. This fraction also revealed a single band upon TLC/blot analysis, whereas the residual organic phase lipid did not show any JONES immunoreactivity. Resorcinol staining identifies a ganglioside band comigrating with this immunoblot band. The JONES reactive material in the ganglioside fraction migrates very close to a GM2 standard; however, JONES does not recognize human GM2. Analysis of adult rat retinal gangliosides fails to detect any JONES immunoreactivity. PN7 rat cerebellar gangliosides reveal positive immunoreactivity identical in migration to retinal extracts, but PN7 forebrain gangliosides are negative. Interestingly, JONES fails to cross-react with mouse or chick retina, or bovine gangliosides.

To understand more about the metabolism of the JONES antigen, we have produced a mouse neuroblastoma x rat E15 neuroblast somatic cell hybrid. JONES positive and negative variants of the cell line have been selected by fluorescence activated cell sorting. The JONES reactive ganglioside in the positive subclone shows the same chromatographic properties as that from rat retina, and the JONES unreactive subclone is negative by this method.

The chemical basis for JONES antigenicity is currently being investigated as are the possible functions which this antigen subserves in development.

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309.12 DISPERSED CELL CULTURES OF THE INNER EAR OF THE CHICK EMBRYO. S.H. Hauger and D.K. Morest, U. Conn. Health Center, Farmington, CT 06032.

We are studying interactions between the neurons of the cochlear and vestibular ganglia and their peripheral synaptic targets, the auditory and vestibular sensory epithelial cells (hair cells). In an organ culture system, we previously observed a trophic effect of the otocyst on these neurons (Ard et al., Neurosci. '85). In order to identify the cellular basis for this effect we are studying dispersed cell cultures.

From stage 23-42 embryos we removed basilar papillae, labyrinths, and cochlear and vestibular ganglia, dispersed the cells enzymatically, and plated them on a dried film of rat tail collagen.

Cultures of ganglia and epithelia together thrived for 1-2 mo and contained areas of fibroblastic cells surrounding monolayer islands of other cell types. Usually all the cells in a given island were similar in size, shape, and arrangement, suggesting that they were the same type. It remains to be shown that these include hair cells. Some cells resembled typical cultured epithelial cells (flat or slightly rounded, polygonal, cobblestone appearance). To verify the identification of epithelial cells we have used histochemical labeling with an antiserum against cytokeratins (Miles, Tissue Tek). Some, but not all of the putative epithelial cells stained, but the antibodies may not be specific for all cytokeratins.

In the foregoing cultures few cochleovestibular neurons were found. However, our growth conditions supported neurons dissociated from trigeminal and spinal ganglia very well. A variety of media, sera, and additives did not improve cochleovestibular neuron survival. This may reflect the lack of a specific factor needed by these neurons.

To enhance neuronal survival, dissociated cochlear and vestibular ganglia were added to pre-plated cochleovestibular cultures without neurons. Neuronal survival was slightly improved. After 1-2 wk the neurons had processes, eucentric nuclei, and Nissl substance. Some cells were notable for resembling cochlear neurons in situ. These were small and bipolar, with one short process and the other much longer, like the peripheral and central processes, resp., of cochlear ganglion cells. Studies in progress are aimed at identifying cultured hair cells with immunohistochemical and ultrastructural methods and at analyzing their interactions with sensory neurons.

(Supported by NIH grant R01 NS14354.)

309.13 THE RAT BRAIN SPECIFIC GENE 1B236 IS EXPRESSED IN CULTURES OF FETAL BRAIN. Dominique Lenoir*, Elena Battenberg, Floyd E. Bloom and Robert J. Milner*. Research Institute of Scripps Clinic, La Jolla CA 92037

Using recombinant DNA techniques, we have identified a rat brain specific protein, 1B236, which defines a widely distributed neuronal system (Cell 33:671, 1983). The time course of expression of this gene during rat brain development has been determined using both RNA and protein blotting techniques and immunocytochemistry. We have shown that the 1B236 gene is expressed postnatally, first in hindbrain around day P5, and then follows a caudal to rostral developmental pattern. The appearance of the protein closely follows that of its corresponding mRNA, suggesting that the developmental expression of 1B236 is probably regulated at the level of transcription. In order to investigate more precisely the characteristics of the regulation of this gene, we have developed a micro-explant culture system: a suspension of minced and triturated tissue fragments from dissected regions (telencephalon, midbrain, cerebellum and hindbrain) of day E19 fetal rat brains was cultured on collagen coated culture dishes. 1B236 protein was first detected after 15 days in vitro in hindbrain cultures using immunocytochemical staining with specific antisera raised against synthetic peptides corresponding to different regions of the protein sequence. Preliminary studies using protein blotting techniques show that the time course of expression of 1B236 in vitro mimics that in vivo: 1B236 protein is expressed earlier in hindbrain cultures than in cerebellum and midbrain cultures. The detection of multiple protein bands on the blot suggests the presence of post-translational modifications such as proteolytic cleavage or differential glycosylation. Further studies will better characterize the time course of appearance of the protein and its mRNA in vitro, the influence of glial cells on gene expression and the nature of the post-translational modifications. The late expression of 1B236 indicates that this protein is a marker for the terminal differentiation of particular neurons. In vitro studies of 1B236 gene expression, therefore, provide a very suitable system to study gene regulation during the final stages of neuronal development. Supported by NIH grant #NS 20728 and AA06420 and grants from McNeil Pharmaceuticals and the Swiss National Foundation.

309.14 IDENTIFICATION OF NEURONAL SUBCLASSES IN DISSOCIATED HYPOTHALAMIC CULTURES. R. Ventimiglia* and H. M. Geller. Dept. Pharmacology, Rutgers Medical School, Piscataway, N. J. 08854, and MRC Neuroimmunology Project, Dept. Zoology, University College, London.

In order to facilitate studies of the cell biology of the hypothalamus, we have developed a technique for the culture of dissociated embryonic neurons in serum-free conditions, and have applied immunological techniques to identify the cell types in these cultures. The procedure which gave optimal results involved culture of hypothalami dissected from 16-18 day embryonic rat brains. The tissue was incubated in MEM-Hepes containing 0.05% trypsin and 0.1% collagenase for 15 min at 37 °C. Following removal of the incubation medium and addition of soybean trypsin inhibitor and DNAase, the tissue was triturated 10X with a Pasteur pipette. Attempts to disscociate by other enzymatic means (dispase or trypsin alone) or solely by mechanical disruption, proved unsuccessful. Cells were resuspended in serum-free N2 medium, and $10^4$ cells were plated onto an astrocyte monolayer growing on 13 mm glass coverslips in 24 well tissue culture plates. Cells adhered to the astrocyte monolayer and extended neuritic processes within 1 hr. after plating. Neurons were identified by staining of neurofilaments with monoclonal antibody RT97 (J. Wood, Sandoz Institute) as early as 1 day after plating. In cultures in which the astrocytes had not formed a confluent monolayer, neurons preferentially aligned along the astrocyte layer. When plated on coverslips coated with collagen, laminin, or polylysine alone, cells adhered poorly and showed little neuritic outgrowth. Hypothalamic neurons could be maintained in serum-free medium for as long as 5 weeks. The majority of neurons were 5-15 μM in diameter and classified morphologically as ovoid-bipolar or triangular-multipolar. Neurons impaled after 8 days in culture displayed both $Ca^{2+}$- and $Na^{+}$-dependent action potentials (B. Fulton, personal communication). In an attempt to identify cell-surface markers for neurons in these cultures, we conducted double-label experiments. A monoclonal antibody produced in the Neuroimmunology Unit stained the surface of the vast majority of cells displaying neuronal morphology, but did not label cells which stained with antibodies directed against GFAP or galactocerebroside. A large proportion of neurons which stained with RT97 were also labelled on their surface by monoclonal antibody A2B5 (Eisenbarth, et. al., 1979). Furthermore, neurons reacting with the vasopressin-specific monoclonal antibody BA-2 (M. Hanley, Imperial College) were detected after 4 weeks in culture. Our results indicate that subclasses of hypothalamic neurons in dissociated cultures can be identified using a variety of intracellular and extracellular markers. Growth of these neurons in serum-free conditions provides a system for the study of this brain region at the single cell level. (Supported by N.S.F. and N.I.H, and a Senior Fellowship of the Fogarty International Center, N. I. H.).

309.15 NEUROGENIC DIFFERENTIATION OF PERIOCULAR MESENCHYME CO-CULTURED WITH GUT. Linda C. Smith-Thomas*, Jeffery P. Davis*, Miles L. Epstein. Department of Anatomy, University of Wisconsin, Madison, WI 53706.

During vertebrate development, cranial neural crest (NC) cells migrate extensively within the developing head and give rise to much of the cranial and facial skeleton (LeLievre, 1978, J. Embryol. exp. Morph., 47, 17-38; Morriss & Thorogood, 1978, Development in Mammals, Vol. 3, North-Holland Publishing Company; Noden, 1978, Dev. Biol., 69, 296-312). In avian embryos, mesencephalic NC cells migrate to the optic cup by 52 hrs of incubation (St 14 - Hamburger & Hamilton) and condense to form the periocular mesenchyme. An interaction between these cells and the retinal pigmented epithelium (RPE) then takes place and as a result of this interaction, the periocular mesenchyme cells become committed to chondrogenic differentiation by 3.5 to 4 days (H.H. St 19-22) (Newsome, 1972, Dev. Biol., 27, 575-579). However, the actual onset of chondrogenesis is not until Day 7 (Romanoff, 1960, 'The Avian Embryo,' MacMillan), indicating that the RPE influence is not required to maintain the chondrogenic potential of the ectomesenchyme.

In order to investigate the influence of the gut environment on periocular mesenchyme (POC), explants of HH19-22 quail POC were co-cultured with chick 4.5 to 5.5 day aneural rectum on the chorioallantoic membrane for 7-8 days. Explants were fixed and sectioned on a cryostat. Slides containing sections were incubated with antiserum to vasoactive intestinal peptide (VIP), monoclonal antibody to neurofilament protein (NF), and bisbenzimide. Using the fluorescence microscope we observed many quail cells, visualized by the fluorescence of bisbenzimide, in the positions usually occupied by enteric ganglia. Many of these quail cells displayed VIP-like immunoreactivity and most showed NF-like immunoreactivity. These results indicated that HH St 19-22 POC cells were able to express neuronal phenotypes. Quail cartilage cells and melanocytes were also observed in the grafts.

These observations suggest that 1) the fate of cells committed to chondrogenesis may be changed to neurogenesis, and/or 2) a population of uncommitted cells within the population committed to chondrogenesis may be directed by the gut environment to form neurons.

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309.16 VENTROBASAL THALAMUS IN INFANT RATS: EFFECTS OF TRANSPLANTATION AND PERIPHERAL RECEPTOR DAMAGE ON ACETYLCHOLINESTERASE-DEPENDENT STAINING. D.A. Kristt, Univ. of Maryland Sch. of Med., Baltimore, Md. 21201

The neurons of the ventrobasal complex (VB) of the thalamus of infant rat and mouse possess high AChE reactivity transiently during their maturation. Intracellular reaction product is detected from 19 days of gestation until 16-18 days postnatal. These neurons synthesize AChE, and resynthesize it after a challenge with an anti-cholinesterase, DFP. The purpose of the present experiments was to investigate two further points regarding the transient AChE phenomenon. The first question was whether the production of AChE by VB neurons was dependent on the localization of the cells to VB. To examine this issue VB from newborn rat pups was transplanted onto the frontal lobe of litter-mates. Following deep cold-anesthesia a pup was decapitated and the brain placed on a chilled platform where it was rapidly sectioned. A 500um slice was produced that contained the VB. The nucleus could be recognized because it was pinker than surrounding regions. The tissue was placed into a small excavation near the frontal pole of the host. The host was sacrificed at 6 dpn. The site of the implant was densely reactive for AChE. The VB implant contained many neurons densely reactive for AChE, while the remainder of frontal cortex contained relatively few reactive cells or fibers. These findings suggest that cellular environment of the VB is not essential for AChE synthesis to occur. In the second experiment the large mystacial vibrissae - tactile sensory organs - on one side of the face were denervated by undercutting the vibrissal pad neonatally. This resulted in total inactivity of the vibrissae on the lesioned side, and hyperactivity of the intact pad. Alterations in the pattern of staining normally present in VB occurred on both sides of the thalamus, although each VB nucleus was affected in a different way. It must be emphasized that the histochemical protocol in use always produced a consistent pattern of staining in VB. At 6 days of age VBM is uniformly more hyporeactive than VBL (Neuroscience 10:923 '83). The experimentally-induced alterations are not likely to be a histochemical artifact. At 6 dpn, VBM showed increased reactivity ipsilateral to the denervated vibrissae, i.e., both subdivisions were equally intensely stained. This may reflect increased activity of the intact vibrissal pad. Contralateral VB showed a focus of strikingly decreased reactivity limited to the vibrissal representation area of VBM. These data suggest that the functional status of the sensory periphery influences the reactivity, and probably the synthesis, of AChE in VB.

In aggregate all of these results indicate that the synthesis of AChE is an intrinsic characteristic of immature VB neurons that is subject to dynamic regulation. Support: NIH, NSF

309.17 GANGLIOSIDE GLYCOSYLTRANSFERASES ARE REGULATED DURING DIFFERENTIATION IN NG 108-15 CELLS. K.M. Walton and R.L. Schnaar, Depts. of Pharmacology and Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21205

Gangliosides are present in high concentrations in nervous tissue and may be important in neuronal cell function. Expression of individual ganglioside species is regulated in developing mammalian brain and upon transformation in many cell types. This laboratory has previously published data on a cell model for this regulation using NG 108-15 neuroblastoma x glioma hybrid cells (Dahms, N.M., and Schnaar, R.L. (1983) J. Neurosci. 3, 806-817). This cell line has four major gangliosides ($GD_{1a}$, $GM_1$, $GM_2$, and $GM_3$) and can be differentiated (as defined by increased choline acetyltransferse activity) by increased cell density or increased intracellular cyclic AMP levels. Upon differentiation, the amount of $GM_2$ (per mg protein) increases up to 12-fold over control levels. We have extended this work by examining the effects of cell density and differentiating agents on the glycosyltransferases responsible for $GM_2$ metabolism.

We developed a rapid and simple assay for UDP-GalNAc:$GM_3$ N-acetylgalactosaminyltransferase ($GM_2$ synthetase) using small DEAE-Sepharose columns. After incubation of NG 108-15 cell homogenates with $GM_3$, UDP-[$^3$H]GalNAc, dAMP, $MnCl_2$, and detergents in MES buffer, the reaction was stopped by addition of chloroform/methanol (2/1) and the mixture applied to a 0.5 ml column of the resin (acetate form). The column was washed with 2 ml methanol, the radiolabeled ganglioside eluted with 4 ml 10 mM potassium acetate in methanol, scintillant added directly and radioactivity determined.

Using this assay, we examined the activity of $GM_2$ synthetase in control cells and cells grown with 1 mM butyrate (a differentiating agent). Within two days after exposure, the activity of the enzyme increased over three-fold above control levels and reached a maximum of a six-fold increase after four days. Within four days, the amount of $GM_2$ (per mg protein) increased over eight-fold, and the levels of $GM_3$ decreased by three-fold. The levels of the other two gangliosides remained constant.

These data demonstrate a direct relationship between differentiation, an increase in $GM_2$ synthetase activity, and $GM_2$ expression in butyrate-treated NG 108-15 cells. In addition, these data indicate there may be coordinate control of the synthetases and/or glycosidases responsible for the metabolism of the other gangliosides which do not change concentration during differentiation. Supported by grants HD 14010 and GM 07626.

309.18 A MONOCLONAL ANTIBODY THAT RECOGNIZES A SUBPOPULATION OF ASTROCYTES IN CULTURE. S.David and T. Crossfield-Kunze*. Neurosciences Unit, The Montreal General Hospital Research Institute, and McGill University, 1650 Cedar Ave., Montreal, Canada, H3G 1A4.

It is possible to identify 2 morphologically different types of glial fibrillary acidic protein positive ($GFAP^+$) astrocytes in culture: (i), large, flat cells, and (ii), small, process-bearing cells. Recent studies indicate that these 2 types of astrocytes also differ in their ability to bind 2 cell surface ligands (Raff et al. J. Neurosci. 3: 1289, 1983). The small, process-bearing (type 2) astrocytes bind the monoclonal antibody A2B5 and tetanus toxin, whereas the large, flat (type 1) astrocytes do not bind these 2 ligands. We have now generated a monoclonal antibody (2.6C6) which recognizes a subpopulation of type 1 astrocytes in culture.

This monoclonal antibody was generated using $E_{19}$ corpus callosum as the immunogen. In a double indirect immunofluorescence assay on dissociated cultures of the neonatal rat cerebral cortex (including the corpus callosum), this monoclonal antibody binds to a cell surface antigen on approximately 35% of the large, flat, $GFAP^+$ astrocytes. It does not label the small, process-bearing astrocytes or any other cell type in these cultures. In frozen sections of brains from $E_{20}$ to adult rats, fixed in 4% paraformaldehyde this monoclonal antibody labels cells predominantly in the white matter, e.g. corpus callosum and optic nerve.

Developmental changes in this antigen were quantified by an enzyme linked immunosorbent assay in which homogenates of cerebral cortex from rats aged $E_{20}$, $P_4$, $P_{11}$, $P_{18}$, $P_{25}$ and adult were plated on polylysine coated microwells (100 µg protein/well). The binding of the monoclonal antibody 2.6C6 to the homogenates was then visualized using a secondary antimouse immunoglobulin conjugated to alkaline phosphatase. Preliminary results show no change in this antigen during development.

Experiments in which dissociated cerebral cortical cultures were incubated with either trypsin or neuraminidase indicate that this antigen is sensitive to trypsin but not to neuraminidase. (Supported by grants from the Canadian MRC and the Spinal Cord Research Foundation).

309.19 A DEVELOPMENTALLY REGULATED MONOCLONAL ANTIBODY TO THE CELL SURFACE OF NEURONS IN THE FOREBRAIN OF THE MOUSE. N.L. Baumrind, J.P. Cohen* and A.L. Pearlman. Departments of Cell Biology and Neurology, Washington Univ. Sch. of Med., St. Louis, MO 63110

During development, neurons destined to comprise the cerebral cortex divide in the ventricular zone, then migrate outward through the intermediate zone to the developing cortical plate. In order to identify cell surface markers specific to the stages of this process, monoclonal antibodies to developing mouse cerebral cortex were generated by fusing Sp2/0-Ag14 mouse myeloma cells with the spleen of a rat immunized against cells taken from the cerebral cortex of mice on embryonic day 13 (E13).

A monoclonal antibody (2A1) was obtained which labels cell bodies and processes of cells located outside the ventricular zone but does not label cells still within it. On paraformaldehyde fixed cryostat sections, 2A1 labels antigenic sites present in the mouse as early as E13. At this time, it labels the region which is about to become the cortical plate. At E15, the antibody labels the marginal zone and intermediate zone, regions containing neuronal fibers. Labelling surrounds cells of the developing cortical plate, but is excluded from the ventricular zone where cells are undergoing division. As development proceeds, newly formed fiber tracts such as the corpus callosum exhibit bright staining. Labelling persists in the adult. At all ages observed, cell membranes of the epithelium of the choroid plexus label strongly.

The 2A1 antibody also labels cell bodies and long processes of unfixed, unpermeabilized, cultured cortical cells, supporting the idea that the antigen is located on the cell surface. Labelled cells have morphologies characteristic of cultured neurons. Double-labelling indicates that 2A1 labels most but not all tetanus-toxin positive cells and most but not all A2B5 positive cells, but does not label cells that are labelled by astrocyte-specific antibodies to GFAP. These observations suggest that the 2A1 antibody is directed against a cell-surface component of neurons that have completed their last division and migrated out of the ventricular zone.

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309.20 MONOCLONAL ANTIBODY TO HUMAN KIDNEY GLOMERULAR BASEMENT MEMBRANE IMMUNOCYTOCHEMICALLY STAINS PERIVASCULAR ASTROCYTES. D.R. Buskirk and M. Hadjiargyrou* The Rockefeller University, New York, NY 10021

Human glomerular basement membrane, obtained from Dr. Howard Fillit, was used to immunize Balb/c mice. After boosting, the splenocytes were fused with NS-1 myeloma cells using standard procedures.

Resulting hybridomas were screened for production of antibodies reactive to kidney by immunofluorescent staining of cryostat sections. Positive hybridoma clones were tested for reactivity to brain, once again by immunofluorescent staining of cryostat sections. One positive clone was identified which reacted with astrocytes in human cerebral cortex. This monoclonal antibody, designated III-70A, stained perivascular astrocytes and their processes. Also stained were white matter astrocytes, but this staining was less intense.

Monoclonal III-70A immunocytochemically stains cell nuclei as well as glomerular basement membrane and astrocytes. The antigen is not known, but has been determined not to be double-stranded DNA both by ELISA and by the antibody's failure to stain the kinetoplast of Crithidia luciliae.

The antibody was found to react with brain sections from a number of different species, including man, cow, rat, and mouse. In each, both perivascular astrocytes and nuclei were stained. However, at higher dilutions of antibody, the astrocytic staining in rats and mice disappeared while the nuclear staining persisted. In man, the antibody titers for both were approximately equivalent.

Although the staining of the cerebral cortex seemed to suggest that the nuclear staining was uniform among cell types, observation of the cerebellar cortex revealed that this was not the case. Although cells of the molecular layer and granule cells stained, the nuclei of the Purkinje cells did not seem to bind antibody. We dot not yet know if this represents a true antigenic distinction of the purkinje cells, or whether the same antigen is present in the nuclei of the Purkinje cells but unavailable for effective immunofluorescent staining.

309.21 CELL SURFACE CHANGES ACCOMPANYING THE NEURAL DIFFERENTIATION OF EMBRYONAL CARCINOMA CELLS. Joel M. Levine* and Patricia Flynn*, (SPON:C.Phelps) Dept. of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, New York, 11794.

Murine embryonal carcinoma cell lines can differentiate in vitro into neuron-like cells. To characterize the cell types present in the differentiating cultures, we have analyzed the expression of cell surface ganglioside and glycolipid antigens during the retinoic acid-induced differentiation of 01A1 cells.

The following antibodies and toxins were used to identify cell surface molecules: monoclonal antibody D1.1 directed against an O-acetylated ganglioside, antibody R24 directed against GD3 ganglioside (both of these antibodies bind to neuroepithelial cells but not to mature neurons or glial cells), antibody SSEA-1 which recognizes a glycolipid determinant present on many undifferentiated EC cells lines and on cells of the developing neural tube, antibody A2B5 which recognizes a GQ ganglioside and labels neurons and glial cells, cholera toxin which binds to GM1 ganglioside and tetanus toxin which binds to GD1b and GT1 gangliosides and is a cell surface marker for most neurons.

Undifferentiated 01A1 cells had the following phenotype: greater than 90% of the cells bound anti-D1.1, R24, SSEA-1, tetanus toxin and cholera toxin. Approximately 30% of the cells bound antibody A2B5. The cells did not contain neurofilament polypeptides or GFAP filaments but the cells were labeled with antibodies against vimentin. Thus, the phenotype of the undifferentiated cells resembles that of neuroepithelial cells.

To induce neuronal differentiation, the cells were grown for 3 days as aggregates in DME containing 10% fetal calf serum and $5x10^{-7}$M retinoic acid (RA). Following 2 days of culture as aggregates without RA, the aggregates were dissociated with trypsin and the cells were plated into defined media containing 5 µg/ml fibronectin. Within 1-2 hours, the cells began to put out processes and after 3-5 days in culture, greater than 80% of the cells were labeled with antibodies against the neurofilament triplet polypeptides. The neurons in the differentiated cultures no longer bound anti-D1.1 or R24 but continued to bind tetanus toxin and cholera toxin. Between 20 and 40% of the cells were labeled with A2B5 and less than 1% of the cells were labeled with SSEA-1. The cell surface changes deduced by antibody labeling studies were also evident when the cells were metabolically labeled with 3-H sugars and cellular gangliosides analyzed by TLC and fluorography. Thus, as 01A1 cells differentiate, they lose ganglioside antigens associated with primitive neurectodermal cells but continue to express surface markers characteristic of neurons and glial cells. These cell lines therefore provide a valuable model system for analyzing the function and metabolism of gangliosides during neuronal differentiation.

309.22 PRODUCTION OF MBP+ AND GC+ MEMBRANOUS SHEETS BY OLIGODENDROCYTES IN VITRO. P.E. Knapp, W.P. Bartlett and R.P. Skoff. Dept. of Anat. Wayne State University School of Med., Detroit, MI 48201.

The primary role of oligodendrocytes is to produce myelin. A fundamental step in this process is the elaboration by oligodendrocytes of large amounts of membrane which expand while wrapping around axons. This ability to produce vast expanses of membrane from the ends of cellular processes is unique in the CNS to the oligodendrocyte. The factors which affect production of oligodendrocyte membranes may be unrelated to the signal to commence myelination, which is known to reside in the axon. We have developed a culture system in which oligodendroglia produce large, membranous expansions in the absence of neurons. Using monoclonal antibodies against galactocerebroside (GC) and myelin basic protein (MBP) we describe their development in vitro.

Cerebra from 3d old mice were used to produce mixed brain cell cultures which were grown in 10% fetal calf serum or in a defined medium. Few neurons survive after one week under these conditions. In 10% fetal calf serum the first GC+ cells appear at 3-4 days in vitro (DIV). Their numbers increase and plateau between 14-21 DIV. At 7 DIV approximately 25% of all GC+ cells have processes which have begun to elaborate sheets. Immunolabeled sheets gradually develop as flattened expansions along the length of cylindrical processes. By 17 DIV as many as 70% of GC+ cells have produced these sheets, which are usually round to ellipsoid in shape, reaching diameters of 80-100u. In older cultures an oligodendrocyte may maintain multiple sheet-bearing processes. Preliminary studies combining (3H)-thymidine autoradiography with immunocytochemistry suggest that a minimum of 4d is required after final cell division before production of GC+ sheets begins. MBP+ cells are first seen by 7 DIV and their numbers peak by day 17. Small MBP+ sheets are not seen until after 12 DIV. Between 17-24 DIV, 50-60% of MBP+ cells elaborate sheets. In mature, double-labeled cells the patterns of GC and MBP staining are identical. When brain cell cultures are maintained in a serum-free, defined medium, considerably greater numbers of GC+ and MBP+ cells and sheets are found as compared to cultures grown in serum. In general the maximal percentages of immunolabeled cells with sheets are similar to those seen in serum supplemented cultures although peak percentages are attained 5-7d earlier. The size and shape of these sheets do not differ from those produced by oligodendroglia grown in 10% serum.

Our in vitro observations suggest that the continued presence of neurons is not required for oligodendrocytes to insert GC and MBP into elaborate membrane structures which mimic the unique and complex morphology of oligodendrocytes in vivo. Thus, the process of myelin membrane production, at least in vitro, may be under regulatory mechanisms which are independant of those controlling axonal ensheathment. Supported by NS 15338.

309.23 FAILURE OF OLIGODENDROCYTES FROM JIMPY BRAIN TO DIFFERENTIATE IN VITRO. W.P. Bartlett, P.E. Knapp and R.P. Skoff. Dept. of Anat., Wayne State Univ. School of Medicine, Detroit, MI 48201

Jimpy (jp) is a sex-linked recessive mutation which produces severe hypomyelination throughout the central nervous system (CNS). Biochemical and morphological studies have shown that oligodendrocytes in jp CNS do not produce normal amounts of myelin. Recent observations from our laboratory (Knapp, et al., this Vol). have shown that normal oligodendrocytes can produce large sheets of galactocerebroside (GC) and myelin basic protein (MBP) positive membranes when grown in vitro. In the present study we compare the differentiation of jp and normal oligodendrocytes grown in vitro for up to 24 days, using monoclonal antibodies to GC and MBP.

Mixed brain cell cultures from 3d mouse cerebra were grown in the presence of 10% fetal calf serum. Two morphologically distinct cell types are recognizable in these cultures by 7-10 days in vitro (DIV). Flattened cells, most of which stain for glial fibrillary acidic protein, form a monolayer on the surface of the coverslip. Small, dark, process-bearing cells (PBCs) lie on the surface of this bed layer and normally comprise 10% of the total cell population at this age. While not all PBCs are stained with GC or MBP, immunostaining is confined to cells with this morphology. Coverslips were prefixed in 4% paraformaldehyde for staining with MBP; GC staining was done on live cells postfixed in 4% paraformaldehyde.

The first GC+ cells are seen in both jp and normal cultures by 7 DIV. However, there are 50-90% fewer GC+ cells in jp cultures than in control cultures at all ages examined. In normal cultures, by 7 DIV approximately 25% of all GC+ cells have begun making sheets. In contrast, less than 5% of the GC+ cells in jp cultures have sheets. This percentage does not increase with time. MBP+ cells are rarely seen in jp cultures. Under phase contrast optics, numerous small, rounded-up cells lying on the surface of the bed layer become apparent in jp cultures by 12 DIV. These cells stain with propidium iodide, a fluorescent dye which accumulates in the nuclei of dead cells. Unlike normal cultures, the numbers of these dead cells in jp cultures increases dramatically as the population of PBCs declines. Since the bed layer remains intact, these dead cells probably derive from small, process-bearing cells lying on the surface.

Our results suggest that the gross deficits in expression of GC and MBP and membrane production by jp oligodendroglia in vitro may be due to their premature death. This parallels our previous in vivo observations of increased oligodendroglial cells death in jimpy CNS (Knapp and Skoff, Am. Soc. Neurosci. Abstr. 10(1):428). Supported by NS 15338.

310.1 ALTERATIONS IN OPIATE RECEPTOR OCCUPANCY FOLLOWING AMYGDALOID KINDLING. S. Weiss*, T. Seeger*, N. Ostrowski, R. Post and A. Pert. Biological Psychiatry Branch, NIMH, Bethesda, MD 20205.

Kindling is a process in which repeated intermittent subthreshold stimulation eventually produces a suprathreshold electrophysiological (and behavioral) convulsive response. Kindled convulsive episodes are followed by a post-ictal period of behavioral depression, the duration and severity of which can be attenuated by prior treatment with the opiate antagonist, naloxone. This and other lines of evidence suggest that opioid peptides are released centrally during the ictal process, and may play a protective role against the immediate repetition of the event. We have employed an in vivo autoradiographic technique which allows the visualization of opioid peptide release in the intact behaving rat (Seeger et al, Brain Res. 305:303, 1984), in order to study the pattern of opiate involvement in the ictal and post-ictal state.

Four adult male Sprague-Dawley rats were implanted with bipolar stimulating electrodes aimed at the central amygdaloid nucleus. The rats were kindled to full tonic-clonic (Stage 5) seizures over a period of three months, and then maintained seizure-free for a further three months. One kindling test session was given to insure sensitivity to stimulation, followed by one more week seizure-free. A full, stage 5 seizure was then induced, which was immediately followed by i.v. injection of the high affinity opiate antagonist, $^{3}$H-diprenorphine (50 µCI/kg) through a jugular catheter. Following a 20 min. wait to allow for washout of the label from non-specific sites, the animals were decapitated and the brains frozen intact for slicing. Four unkindled control rats with electrodes implanted in the ventral tegmental nucleus were injected identically and matched to kindled rats on the basis of cerebellar (non-specific) binding levels. Comparisons were than made between anatomically equivalent sections from these matched pairs, exposed on the same sheet of LKB Ultrofilm. Film analysis was by computer-assisted densitometry.

In the kindled group, acute seizure induced significant bilateral decreases in diprenorphine binding at numerous levels of the forebrain. These included 35-40% decreases in specific binding in cingulate cortex, nucleus accumbens, and dorsal hippocampus, while no significant change was seen in the caudate or motor cortex. At more posterior levels, binding in the basolateral amygdala was decreased by 48%, while smaller decreases (20-30%) were found in the ventral hippocampus, cortical amygdaloid nucleus, hypothalamus and sensory cortex. No change was seen in the thalamus or in any more posterior structures. These results suggest widespread occupation of forebrain opioid receptors by endogenous opiates released during the ictal process which prevented the binding of the exogenous radio-labeled opiate ligand. Alternatively, kindling may have induced region-specific changes in opiate receptor binding characteristics.

310.2 ARCUATE NUCLEUS STIMULATION: INDUCTION OF NALOXONE-REVERSIBLE ANALGESIA WITH CONCOMITANT LOCALIZATION OF OPIATE RELEASE USING IN VIVO AUTORADIOGRAPHY. T.F. Seeger and A. Pert (SPON: N. Bacopoulos) Biological Psychiatry, NIMH, Bethesda, MD 20205

The arcuate nucleus of the hypothalamus is the major source of beta-endorphin efferents in the CNS, suggesting that it may be involved in pain control. We have tested this nucleus for induction of stimulation-induced analgesia, and subsequently monitored the same animals for the release of opiate peptides, using the technique of in vivo autoradiography (Seeger et al, Brain Res. 305:303, 1984).

Eight male Sprague-Dawley rats were implanted with bipolar electrodes into the arcuate nucleus. Following recovery, pain sensitivity was quantified using a hot-plate test (latency to lick rear paw with plate at 55 C). Electrical stimulation (applied for 1 min in 3/sec bursts of 100 msec, 50Hz, 50usec pulsewidth, 100 usec delay) caused a significant increase in latency which lasted beyond the period of stimulation. Threshold current for inducing analgesia was 600 uAmp, with maximal effect at 1-1.3 mAmp. Pretreatment with naloxone (5mg/kg i.p. 15 min before testing) had no effect on baseline latency, but blocked the increase due to stimulation. These arcuate-implanted rats differed from a similarly treated group with electrodes in the periaqueductal grey in which analgesia did not outlast the stimulation and was not naloxone-reversible.

Following analgesia testing, all of the rats were implanted with intravenous jugular catheters for the autoradiography procedure. Electrical stimulation was applied to half of them for one minute, after which each rat received an injection of 3H-diprenorphine (50 uCi/kg) while the stimulation continued. After twenty minutes (to allow washout from non-specific binding sites), the rats were decapitated and the brains frozen intact for slicing. The other four rats were prepared identically, but without any stimulation. Each stimulated rat was matched with a control rat on the basis of cerebellar binding levels. Comparisons were made between anatomically equivalent sections from these matched pairs, which were exposed on the same sheet of 3H-Ultrofilm. Analysis of the film was by computer-assisted densitometry.

Significant bilateral decreases (up to 22%) in diprenorphine binding were found in a number of brain regions, including the anterior and medial amygdala, striatal fundus, bed nucleus, preoptic area, hypothalamus, and periaqueductal grey. No changes were found in basolateral amygdala, medial thalamus, cortex, or white matter. This pattern of changes indicates increased receptor occupancy in terminal projection areas of the arcuate nucleus due to stimulation-induced release of beta-endorphin.

310.3 CALCITONIN INDUCED DEPRESSION OF LOCOMOTOR BEHAVIOR IS MEDIATED THROUGH CALCITONIN RECEPTORS IN THE NUCLEUS ACCUMBENS. H.D. Everist, A. Fabbri*, and A. Pert. Biological Psychiatry Branch, NIMH, Bethesda, Maryland 20205 and Policlinico Umberto I°, Universita di Roma, Rome, Italy.

Calcitonin evokes potent effects on locomotor behaviors. Little, however, is known regarding the specific sites of action of this peptide in modifying locomotor output. In this study we have visualized calcitonin receptor distribution in the rat forebrain with autoradiographic procedures and then evaluated the effects of this peptide on locomotor behavior following direct injections into calcitonin receptor rich areas.

For receptor visualization, several 24 u m sections were taken through the rostral part of the brain. These sections were exposed to $^{125}$I-salmon calcitonin (sCT) and then processed for autoradiography using tritium-sensitive film. Autoradiographic analyses revealed exceptionally heavy binding of sCT in the nucleus accumbens. The caudal portion of the nucleus appeared to have somewhat higher labeling than the rostral segment. Little binding was evident in the caudate nucleus or other forebrain regions. Lesions of the ascending dopamine pathway with 6-OHDA failed to alter sCT binding in the n. accumbens. Injections of kainic acid into the n. accumbens, on the other hand, decreased binding on the lesioned side. These findings suggest that sCT binding sites are located on cell bodies intrinsic to the n. accumbens and not on DA terminals that innervate this region.

In subsequent studies, rats were implanted with chronic indwelling cannulae guides aimed for the lateral ventricles, nucleus accumbens or caudate nucleus. Intraventicular injections of sCT (1, 10 or 25 n moles) produced a dose-dependent long lasting decrease in locomotor behavior. Bilateral injections of sCT into the n. accumbens resulted in an even more dramatic response. As little as 0.02 n moles decreased horizontal locomotor activity by 53% in the first 15 minutes. Higher doses (0.1-2.0 n moles) had an even greater effect. Injections of 0.5 n moles into the caudate nucleus, on the other hand, had little effect on locomotor output.

These findings indicate that sCT is an extremely potent modulator of locomotor output. The effect appears to be mediated predominantly through the interaction of this peptide with sCT binding sites on neurons intrinsic to the n. accumbens.

310.4 NALOXONAZINE: EFFECTS ON FOOD INTAKE AND CONDITIONED TASTE AVERSION. D.L. DeHaven, P. Brostrom* and L.R. Steranka. Nova Pharmaceutical Corporation, 5210 Eastern Avenue, Baltimore, MD 21224

Naloxonazine (NAZ) has been reported to be a selective antagonist at mu opioid receptors and to inhibit feeding in rats (Hahn et al., J. Neurosci. 2: 572, 1982; Simone et al., Life Sci. 36: 829, 1985). The present series of experiments attempted to further evaluate the anorectic properties of NAZ and to determine whether the effects of NAZ on feeding might be attributable to the production of a conditioned taste aversion (CTA). In the first experiment, after establishment of a stable baseline for feeding, male Sprague-Dawley rats were food deprived for a 24 hr period immediately before injection of NAZ (2, 5, 10 or 20 mg/kg SC) or vehicle. Food and water intakes were recorded at 1, 2 and 24 hr after treatment. All doses of NAZ effectively inhibited feeding for up to 2 hr after administration, with food intake of treated rats returning to control levels by 24 hr. Water consumption of NAZ-treated rats was also slightly decreased at 1 and 2 hr, but not at 24 hr. The second experiment assessed the ability of the above doses of NAZ to produce a CTA. Animals were conditioned to the aversive properties of LiCl or NAZ by the procedure of Wagner et al. (Pharmacol. Biochem. Behav. 14: 85, 1981), which utilizes a two-bottle choice on the test day. NAZ at the 20 mg/kg dose produced a CTA equivalent to that seen with LiCl (12.7 mg/kg), while 10 mg/kg NAZ produced a slight decrease in the preference ratio of milk intake/milk + water intake without affecting the total amount of milk consumed. NAZ in doses of 2 and 5 mg/kg had no effect. These data suggest that although the production of a CTA by higher doses of NAZ may affect feeding, lower doses of NAZ have pure anorectic properties. Therefore, NAZ may have potential use as an appetite suppressant.

310.5 AN ENKEPHALIN-LIKE PENTAPEPTIDE (BW942C) WITH PARTIAL KAPPA AGONIST ACTIVITY. D.B. Vaupel*, E.J. Cone* and R.E. Johnson* (SPON: L. Porrino). NIDA Addiction Research Center, Baltimore, MD 21224.

BW942C (Tyr-D-Met(O)-Gly-pNO$_2$-Phe-Pro-NH$_2$) is a pentapeptide that has a morphine-like antidiarrheal effect in the rat. Compared to morphine or loperamide, BW942C was more potent and thus showed selectivity as an antidiarrheal agent. Human pharmacological studies at the Addiction Research Center suggested that this peptide may have opioid activity of the kappa-type. For this reason, BW942C was evaluated in the rat urination model which measures the diuretic effects of opioids having kappa- or ketocyclazocine-type activity and antidiuretic effects of opioids with mu or morphine-type activity. Urinary output was determined in normally fed and hydrated, adult male Long-Evans hooded rats placed in metabolic cages. Urine volumes were measured at 2 and 5 hr following s.c. drug or vehicle administration. Six rats were tested at each treatment condition. BW942C (0.01 to 3 mg/kg) was compared to bremazocine (0.0008 to 0.08 mg/kg) and U-50,488H (0.01 to 30 mg/kg), two compounds which exhibit activity of the kappa type. Dose-response curves demonstrated that both bremazocine and U-50,488H were full agonists with peak urine outputs approaching 20 ml over 5 hrs. The water vehicle treated animals produced 3 ml of urine over 5 hrs. BW942C produced a biphasic dose-response curve. Doses of 0.01 to 0.3 mg/kg doubled the urine output from 3 to 6 ml, whereas 1 mg/kg had no effect, and 3 mg/kg completely suppressed urine flow. Thus, BW942C appeared to be a partial kappa agonist at low doses and exhibited mu or morphine-type activity at higher doses in the rat urination model. Most of the BW942C-induced diuresis occurred within the first 2 hrs. In parallel line bioassays, BW942C was 0.1 and U-50,4884 was 0.004 times as potent as bremazocine. In antagonism experiments, naltrexone (0.01 to 0.1 mg/kg) reversed the antidiuretic effect of 3 mg/kg of BW942C to reveal a diuretic effect; higher doses of naltrexone (1 and 10 mg/kg) were required to antagonize this diuresis. The diuretic effects of intermediate doses of bremazocine (0.0132 mg/kg) and U-50,488H (3.89 mg/kg) also were antagonized by the simultaneous administration of BW942C in increasing doses. Maximally effective doses of the full agonists were more difficult to antagonize by BW942C. These data are consistent with the interpretation that BW942C is a weak partial kappa agonist over a limited dose range. This is the first report of a peptide that has activity characteristic of a partial agonist at the kappa opioid receptor. The diuretic activity of BW942C suggests that it has a relatively low intrinsic activity for the kappa receptor which is in part self-limited by its mu or morphine-like activity which emerges at higher doses.

310.6 HIGHLY SELECTIVE MU- AND DELTA- OPIOID AGONISTS AND ANTAGONISTS: ACTION ON SENSORY NEURON CALCIUM-DEPENDENT ACTION POTENTIALS. R.L. Macdonald, M.A. Werz, and D.S. Grega. Dept. of Neurology, Univ. of Michigan, Ann Arbor, MI 48104.

We have previously reported that a subpopulation of DRG neurons grown in primary dissociated cell culture have mu- and/or delta- opioid receptors on their somatic membrane and that binding of opioid ligands to these receptors results in a decrease of calcium-dependent action potential duration (Werz and Macdonald, JPET, 227:394-402, 1983). In the present investigation we have assessed agonists and antagonists with high selectivity for either mu- or delta-receptors. The majority of DRG neurons did not respond to 1 μM [N-MePhe$^3$, D-Pro$^4$]-morphiceptin (MC), a selective mu- ligand, 1 μM [D-Pen$^2$, L-Pen$^5$]-enkephalin (DPLPE), a selective delta-ligand, or 1 μM leucine-enkephalin (L-ENK), a non-selective ligand. However, a small population of DRG neurons responded to both MC and L-ENK but not DPLPE and another small population responded to both DPLPE and L-ENK but not MC. These data are consistent with MC selectively marking neurons with mu- receptors, DPLPE selectively marking neurons with delta-receptors, and L-ENK not distinguishing between these receptor types. We have further characterized responses of DRG neurons to MC with selective antagonists. Responses to 1 μM MC were antagonized by naloxone (dose-dependently over 100 pM-10nM) and also by the somatostatin analog SMS 201-995 (dose-dependently over 1nM-100nM). The selective delta antagonist ICI 174864 was without effect at 10 μM. The irreversible ligands β-funaltrexamine (β-FNA) and naloxonazine (NZ) were also investigated. Ten to 20 neurons within a culture were impaled. The response to MC assessed and the location of neurons responding to MC was noted. The culture was then incubated in β-FNA or NZ at 37°C for 1 hr. Following extensive rinsing, the neurons that had responded to MC previously were reimpaled. Both β-FNA and NZ at 250 nM irreversibly reduced responses to MC by 76%. β-FNA at 1 nM did not affect responses to MC.

Our data using selective agonists and antagonists confirm that both mu- and delta- receptors are present on DRG neuron somata. Investigations of DRG neurons in culture will provide information of the neuronal membrane effects that result following ligand binding to each receptor type.

We thank Dr. Beat Gähwiler for the gift of SMS 201-995, Dr. Hank Mosberg for DPLPE, Dr. Gavril Pasternak for NZ, and Dr. P. Portoghese for β-FNA.

310.7 THE EFFECTS OF N-METHYLMORPHINE ON THE RELEASE OF ANTERIOR PITUITARY HORMONES. R.N. Pechnick, R.E. Poland* and R. George*. Department of Pharmacology, The Brain Research Institute and the Laboratory of Neuroendocrinology, UCLA, Los Angeles, CA 90024 and Division of Biological Psychiatry, Harbor-UCLA Medical Center, Torrence, CA 90502.

The acute administration of morphine sulfate elicits dose-dependent increases in the serum levels of ACTH, GH, and PRL while inducing decreases in LH and TSH. The locus of this action of the opiates is thought to be at the level of the hypothalamus, presumably by affecting the release of hypothalamic releasing inhibiting factors. There are some data suggesting that systemically administered opiates and opioid peptides can act directly on the pituitary or the median eminence to influence hormone release. To test this hypothesis we examined the effects of N-methylmorphine, a quaternary analogue of morphine that does not cross the blood-brain barrier, on the release of corticosterone, GH, PRL, LH and TSH.

Male rats were injected with N-methylmorphine chloride (20.0 and 40.0 mg/kg s.c.) and trunk blood obtained 30 min later. Serum hormone levels were measured by RIA. Both doses of drug caused increases in the release of GH and PRL; however, serum levels of corticosterone, LH and TSH were unchanged. These results indicate that opiate-induced release of GH and PRL may be mediated in part by sites outside of the blood-brain barrier.

310.8 INHIBITION OF [$^3$H]NOREPINEPHRINE RELEASE FROM RAT AND GUINEA PIG BRAIN SLICES BY OPIOIDS. L.L. Werling, S. R. Brown*, and B.M. Cox. Department of Pharmacology Uniformed Services University, Bethesda, MD 20814.

The locus coeruleus is the origin of noradrenergic fibers which project to several areas of the rat and guinea pig central nervous systems, including the cortex, cerebellum, and the hippocampus. Various lines of electrophysiological and anatomical evidence suggest that the activity of these neurons may be influenced by opioid neurons which impinge upon them, and several groups have reported the inhibition of [$^3$H]norepinephrine ([$^3$H]NE) release by opioids, although none of these has identified the type of opioid receptors which mediates this effect. We have demonstrated that μ, δ, and κ selective compounds all decrease the $K^+$ stimulated, $Ca^{++}$ dependent release of [$^3$H]NE from slices of guinea pig and rat brain tissues, but the tissues exhibited differential sensitivity to these compounds. Release from guinea pig cortex, cerebellar cortex, and hippocampus was depressed to a greater extent by treatment with the κ selective U50,488H than by exposure to the μ selective DAGO. [$^3$H]NE release from rat cortical slices was greatly depressed by DAGO, while U50,488H produced a more modest inhibition. Maximal depression by the δ selective DPDPE was intermediate between that produced by DAGO or U50,488H in both species, but DPDPE had a higher potency in rat cortex than in guinea pig cortex. These results suggest that although norepinephrine release may be regulated by opioids in both guinea pig and rat brain, the receptors which are most effective in regulating release are not identical in each species. Additionally, release of norepinephrine may be regulated by more than one type of opioid receptor.

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310.9 ENDORPHIN BLOCKADE SYNDROME: EFFECTS OF RATE AND DURATION OF NALOXONE INFUSION IN MALE AND FEMALE RATS. R.F. HAMILTON JR. *, D.H. Malin, C. Vandenberg* and R.J. Townsend III.* (Spon: C.D.B. Bridges) Univ. of Houston-Clear Lake, Houston, Texas 77058.

A single injection of naloxone fails to elicit typical opiate abstinence signs in a normal, non-dependent animal. However, a prolonged injection series (Malin et al, Pharmacol. Biochem. Behav. 17:389, 1982) or a day of continuous naloxone infusion (Malin et al, Pharmacol. Biochem. Behav. 22:791, 1985) did induce a number of opiate-abstinence-like signs.

The present experiment was performed to determine how the frequency of abstinence-like symptoms is affected by the length and rate of naloxone infusion and by sex differences. Thirty female and thirty male rats (all 60 days) were divided into five equal infusion rate groups: 0.0, 0.35, 0.7, 1.4, and 2.1 mg/kg/hr naloxone delivered by subcutaneously implanted Alzet 2ML1 osmotic minipumps. Each animal was observed for 10 min. for standard behavioral abstinence signs after 1, 2, and 3 days of infusion.

A 5x2x3 ANOVA with one repeated measures variable revealed significant, $p < .01$, main effects of infusion rate and days of infusion but not of sex. There were no significant interaction effects. The effect is dose-dependent, since abstinence-like signs showed a significant positive linear trend as a function of infusion rate. The effect declined significantly by day 3, despite continued infusion of naloxone, suggesting some sort of dynamic adjustment to endorphin receptor blockade.

The only significant sex difference was at the infusion rate of 0.7 mg/kg/hr, where there was a sharp drop in the abstinence-like response of the female rats. This accounts for our previous failure to find an effect in females, since this was the infusion rate previously employed. (Supported by Univ. of Houston-Clear Lake Organized Research Grant.)

310.10 **A SEIZURE-PRODUCING LESION ENHANCES KAINIC ACID RESPONSIVITY IN THE HIPPOCAMPAL SLICE PREPARATION.** R.M. Pico and C.M. Gall. Dept. of Anatomy, University of California, Irvine, CA 92717.

Recurrent limbic seizure activity induced by a unilateral electrolytic lesion of the mouse dentate gyrus hilus has been found to cause a transient (10-12 day) bilateral increase in mossy fiber enkephalin immunoreactivity and a persistent (>20 day) loss of mossy fiber cholecystokinin immunoreactivity (C. Gall, Neurosci. Abstr., 10, 1112, 1984). We have begun to investigate the physiological consequence of this fluctuation in endogenous peptide levels using the in vitro hippocampal slice preparation. The present study examined the influence of kainic acid (KA) on hippocampal slices from control (untreated) mice and experimental animals sacrificed either 4 or 14 days after contralateral hilus-lesion placement. KA has been reported to induce spontaneous multiple discharges, interictal spikes, in rat hippocampal slices at low concentrations (0.05-1.0uM) (G.L. Westbrook, & E.W. Lothman, Brain Res., 273, 97-100, 1983). Slices (425um) were maintained with a constant perfusion of oxygenated artificial CSF at 34°C. Field potentials were recorded by electrodes placed in CA3 stratum pyramidale. Experimental and paired control slices were simultaneously monitored. With the addition of only 16 nM KA to the media perfusion, 92% of the 4 day postlesion slices had begun to spike (32 total sliced, 5 mice). Most control slices and virtually all experimental slices had interictal spikes at 100nM KA. Examination of 2 mice (11 total slices) lesioned 14 days prior to testing, revealed no difference in the response to KA when compared to 2 control mice (10 total slices). No response was detected at 16 or 25nM KA, while 100nM KA elicited spikes from 66% of the treatment slices and 50% of the control slices.

These results demonstrate a correlation between the hilus lesion-induced increase in mossy fiber enkephalin content and reduced threshold for KA stimulation of epileptiform activity in the hippocampal slice preparation. Pharmacological studies are in progress to determine the contribution of the mossy fiber peptides to this effect.
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310.11 CHANGES IN CENTRAL ADRENERGIC RECEPTOR FUNCTION MAY CONTRIBUTE TO OPIATE DEPENDENCE. H. C. Moises and C. B. Smith.*+ Dept. of Physiology and Dept. of Pharmacology+, The University of Michigan, Ann Arbor, MI 48109.

Considerable evidence indicates that significant components of the opiate withdrawal syndrome may be due to increased central noradrenergic activity in areas such as the locus coeruleus. Our recent findings of increases and decreases in beta and alpha$_2$ adrenergic receptors, respectively, in various brain regions in rats treated chronically with morphine (Neurosci. Abstr. 9:797, 1983) raise the additional possibility that changes in central adrenergic receptor function may contribute to or reinforce such effects. In this study, radioligand binding assays were carried out in conjunction with intracellular recording from hippocampal pyramidal neurons in vitro to determine whether alterations in beta receptor density that occur in hippocampus following chronic morphine treatment are reflected in corresponding changes in postsynaptic sensitivity to noradrenergic stimulation.

Sprague-Dawley rats (175-225g) were injected with increasing doses of morphine (up to 100 mg/kg/injection) or saline, i.p., every 8 h for 14 days. Animals were killed by decapitation either 8 h or 32 h after the last injection and neural membrane preparations and 400 μm thick slices of hippocampus prepared. Slices were maintained at 35.5°C under total submersion and allowed to equilibrate for 1.5 h prior to initiation of drug superfusion and electrophysiological recording. The ability of norepinephrine (NE) to block the late hyperpolarization response of pyramidal neurons to depolarizing current pulses (500 ms, 0.5-1nA) was determined over a range of drug concentrations to assess changes in postsynaptic beta receptor function (Madison and Nicoll, Nature 299:636, 1982).

Following chronic morphine treatment, the $B_{max}$ for binding of $^3$H-dihydroalprenolol ($^3$H-DHA) showed a biphasic change, indicating an initial increase (13.8%, $p<.05$) and subsequent decrease (-26.4%, $p<.005$), relative to controls, in beta receptor number in hippocampus with time during withdrawal. On the other hand, the apparent affinities of the receptors for $^3$H-DHA and for several agonist radioligands were not significantly changed. Electrophysiological testing revealed that the changes in beta receptor density found in early (8 h) and later phases (32 h) of withdrawal were paralleled by a corresponding decrease ($p<.06$) and increase ($p<.01$), respectively, in the $EC_{50}$ for NE in blocking the late hyperpolarization response. These results suggest the possibility that changes in postsynaptic sensitivity to noradrenergic stimulation may be important in the formation or expression of opiate dependence. (Supported by NIDA Grant DA-03365 and The Chicago Community Trust/Searle Scholars Program).

310.12 LOCAL STIMULATION OF GLUCOSE UTILIZATION IN THE SPINAL CORD DURING NALOXONE-PRECIPITATED OPIOID ABSTINENCE. J.A. Bell*, A.S. Kimes and E.D. London (SPON: J.E. Warnick). Neuropharmacology Lab., NIDA Addiction Research Center, Baltimore, Maryland 21224.

The opioid abstinence syndrome is characterized by signs of CNS hyperexcitability. Hyperalgesia is a prominent sign which is distinguished by hyperreactivity to nociceptive stimuli in experimental animals, and by abdominal cramps, and pains in bones and muscles of the back and extremities in humans. Several studies have shown that substance P is associated with neuronal mechanisms concerning nociception in the substantia gelatinosa of the spinal cord.

Previous studies have demonstrated that the spinal release of substance P is inhibited by morphine (Yaksh, T.L., et al., Nature, 286: 155, 1980; Y. Kuraishi et al., Life Sci 33 Suppl 1: 693, 1983). Furthermore, chronic morphine treatment increases levels of substance P in the dorsal horn of the spinal cord (Naftchi, N.E., et al., Peptides Suppl. 1:61, 1981), Bergstrom et al., Life Sci., 35:2375, 1984). Therefore, following abrupt withdrawal of chronic morphine treatment, hyperalgesia could result from hyperactivity of substance P systems in the dorsal horn of the spinal cord. In order to obtain information about the activity of neural pathways in areas of the spinal cord where substance P is active in nociceptive transmission, we studied local spinal glucose utilization (LSGU) during naloxone-precipitated morphine abstinence.

Studies were performed in male Fischer-344 rats which were implanted with one 75 mg morphine or placebo pellet on day 1 and 2 pellets on day 4. LSGU was measured on day 8 by the autoradiographic 2-deoxy-[1-$^{14}$C]glucose ([$^{14}$C]DG) method (Sokoloff L., et al., J. Neurochem. 28:897, 1977) in rats which were partially restrained by lower-body plaster casts, using standard values for the lumped constant and rate constants for the transport and phosphorylation of [$^{14}$C]DG. Naloxone (0.5 mg/kg, s.c.) or saline were injected 2 min before [$^{14}$C]DG (125μ Ci/kg). Rats were killed by an overdose of sodium pentobarbital. The spinal cord was rapidly removed and divided into lumbar, thoracic, and cervical sections, and frozen.

An increase in LSGU was seen in the superficial portion of the dorsal horn in sections of the spinal cord from rats which had received chronic morphine and exhibited marked withdrawal signs following naloxone administration.

These studies indicate that increased metabolic activity, reflected by increased LSGU, occurs in superficial portions of the dorsal horn of the spinal cord corresponding to the location of the substantia gelatinosa. These increases may be associated with a rebound hyperactivity of substance P pathways that could contribute to hyperalgesia during the morphine abstinence syndrome.

310.13 PHYSIOLOGICAL INTERACTIONS OF MORPHINE AND DILTIAZEM IN RATS. M. Szikszay*, G. Benedek* and E.D. London (SPON: D. Jasinski). Neuropharmacology Laboratory, NIDA Addiction Research Center, Baltimore, MD 21224 and Dept. Physiology, Univ. Med. School of Szeged, Hungary.

Calcium channel antagonists (such as diltiazem) can potentiate the antinociceptive and hypothermic effects of morphine in rats (Benedek, G. and Szikszay, M., Pharmacol. Res. Commun., 16:1009, 1984). In order to determine if diltiazem's effect on morphine-induced antinociception is dose-dependent, we measured antinociception by means of hot-plate (53.5°C) and tail-flick (53°C) tests. A subcutaneous injection of 4 mg/kg morphine sulfate caused slight increases of the hot-plate and tail-flick latencies in rats. Simultaneous administration of diltiazem (2, 10 or 20 mg/kg, subcutaneously) caused a dose-dependent potentiation of morphine's antinociceptive effect.

Because it was of interest to determine if such a positive interaction extended to other physiological actions, we studied the effects of morphine and diltiazem alone and in combination on indices of respiratory and cardiovascular function in partially restrained, awake male Fischer-344 rats. A subcutaneous injection of 4 mg/kg morphine sulfate produced hypercapnia, hypoxia and slight acidosis, as compared with values in arterial blood from control rats. Diltiazem alone (10 mg/kg,) did not affect these parameters, but it significantly delayed the times of the peak hypercapnic and hypoxic effects of morphine. Marked hypercapnia and hypoxia were seen at every time point after morphine treatment. Peak hypercapnic effects of morphine and morphine + diltiazem did not differ significantly in magnitude (increases of 5.1 + 0.84 and 4.1 + 0.94 mm Hg, respectively), but diltiazem significantly delayed the onset of the peak effect from 0.38 + 0.06 hr to 1.0 + 0.23 hr, respectively. The maximum effects of morphine and morphine + diltiazem on $Pa_{O_2}$ were of similar magnitude (declines of 17.7 + 2.26 and 15.7 + 2.28 mm Hg, respectively), but diltiazem significantly delayed the time of the peak hypoxic effect of morphine from 0.46 + 0.04 hr to 0.92 + 0.08 hr. Morphine produced a marked elevation of heart rate; whereas, diltiazem alone or in combination with morphine produced a slight tachycardia. Mean arterial blood pressure was not affected by morphine, but diltiazem alone or in combination with morphine reduced blood pressure dramatically (about 25 mm Hg below baseline).

In conclusion, the influence of the morphine-diltiazem interaction on respiration differs from its effect on antinociception and thermoregulation. This observation supports the view that opioid effects on respiration are mediated at different sites than the other aforementioned effects.

310.14 WITHDRAWN

310.15 PARASYMPATHETIC ACTIVITY MEDIATES THE CARDIAC BUT NOT THE VENTILATORY DEPRESSION INDUCED BY ENKEPHALINS IN CONSCIOUS DOGS. G.G. Haddad*and P. Lasala.* (SPON: L. J. Côté ). Departments of Pediatrics (Pulmonary Division) and Neurosurgery, Columbia University, College of Physicians & Surgeons, New York, NY 10032.

We have previously shown that intracisternally (IC) injected enkephalin analogs (DADLE) increase the RR interval and depress ventilation in unanesthetized adult dogs. Because of the known interactions between opioids and the cholinergic system, we asked whether the cardiorespiratory changes induced by DADLE are mediated by the parasympathetic nervous system. Eleven experiments were performed on 4 unanesthetized dogs with chronically implanted cannulae in the Cisterna Magna and aortic catheters. Ventilation was measured non-invasively using barometric plethysmography and the RR interval using a dedicated RR preprocessor and a microcomputer. RR interval and ventilation were recorded continuously and automatically acquired using real time data acquisition techniques. Atropine sulfate was given systemically (20 mcg/kg) at peak action of DADLE (25 or 125 mcg/kg). Approximately 40 minutes post DADLE (peak action), mean RR interval had increased by 49.9% and 92.3%, and mean minute ventilation had decreased by 28.3% and 43.5% at 25 mcg/kg and 125 mcg/kg respectively ($p < 0.05$). $PaCO_2$ increased in every study by 8-14 Torr. Within minutes after the injection of Atropine, mean RR interval decreased to about baseline levels, but mean minute ventilation and $PaCO_2$ did not change. At the end of each DADLE experiment, Naloxone (given IC in doses of 1-10 mcg/kg) increased minute ventilation, reduced $PaCO_2$ and decreased the mean RR interval even further. Atropine or Naloxone had little or no effect (<5%) on cardiorespiratory function when given without prior administration of DADLE. We conclude that the DADLE-induced prolongation of the RR interval is mediated by parasympathetic mechanisms. We speculate that the DADLE-induced ventilatory depression is either due to direct opioid action or to non-cholinergic mediating mechanisms on central respiratory regulation.

310.16 MURAMYL DIPEPTIDE DOES NOT AFFECT OPIOID BINDING IN GUINEA PIG BRAIN. M. S. Ahmed*, J. M. Krueger, and C. M. Blatteis. Univ. of Tenn. Ctr. Hlth. Sci., Memphis, TN 38163.

A muramyl peptide promoting slow-wave sleep and inducing fever has been isolated from the brains of sleep-deprived rabbits. A synthetic immunoadjuvant, muramyl dipeptide (MDP, N-acetylmuramyl-L-alanyl-D-isoglutamine), injected intracerebroventricularly at doses of 50-100 pM, also is somnogenic and pyrogenic. It has been suggested that interleukin 1 (IL1), which possesses similar somnogenic and pyrogenic properties, may mediate these actions of MDP because the production of IL1 by macrophages is stimulated by MDP. Recently, evidence has been provided that IL1 contains an MDP-like structure or MDP binds nonspecifically to IL1. Because IL1 reduces the specific binding of opioid ligands to their receptors in various regions of guinea pig brain, and because of the central role of opioids in immunoregulation and wakefulness, the present study was designed to determine whether MDP shares the capacity of IL1 to interact with opioid receptors. Accordingly, membrane-bound opioid receptor preparations were obtained from the cerebral cortex, hypothalamus, midbrain, pons, and medulla of 12 guinea pigs, in two replications. Aliquots of the membrane homogenates were incubated with the tritiated opioid ligands 2-D-alanine-5-L-methionineamide (DAME) or dihydromorphine (DHM); the volume of the assay mixture was 0.5 ml. Specific opioid binding was calculated as the difference between the counts of radioligand bound in the presence or absence of 1.0 μM levorphanol. MDP at the concentrations of 1.0, 0.2, and 0.02 μM did not affect the specific binding of DAME or DHM in any of these guinea pig brain regions. We conclude, therefore, that the central activities of MDP do not involve any direct interactions with brain opioid receptors. (Supported by NSF grants No. BNS 83-08257 and PCM 83-17217.)

310.17 OPIOID RECEPTORS AND BODY TEMPERATURE IN RATS. M.W. Adler* and E.B. Geller. Dept. of Pharmacology, Temple University School of Medicine, Philadelphia PA 19140.

We have previously reported that subcutaneously (sc) administered opioids can be classified into groups based on their effects (at an ambient temperature of 20°C) on body temperature in rats, response to naloxone, and stereospecificity (Geller et al., J. Pharmacol. Exp. Ther. 225:391, 1983) and that the temperature response to these opioids often differs qualitatively if the drugs are given by the intracerebroventricular (icv) route (Adler et al., Neuropeptides 5:73, 1984). In addition, we have reported on the effects of the endogenous opioid peptides β-endorphin,D-$ala^2$-met-enkephalinamide,D-$ala^2$-D-$leu^5$-enkephalin, and $dynorphin_{17}$ on body temperature in the rat (Adler et al., in: Environment, Drugs, and Thermoregulation, P. Lomax and E. Schönbaum, eds., Karger:Basel, 1983). Based on these and other studies, we have postulated that the thermal actions of the opioid system are explainable by a 2-receptor model. Specifically, μ receptors mediate hyperthermia and κ receptors mediate hypothermia. Further, the μ effects, at least in part, are due to actions in the brain while κ actions are primarily outside of the brain (possibly outside of the CNS). Among the findings in support of this hypothesis are the following: 1)agonists which have predominant activity at the μ receptor produce hyperthermia whether administered sc, icv, or directly into the preoptic anterior hypothalamus (POAH); 2)agonists which have predominant activity at the κ receptor produce hypothermia when administered sc and have either no effect on body temperature when administered by the icv route (e.g., U50,488H, the highly selective κ agonist) or produce a dose-related hyperthermia icv (e.g., EK, which is known to have agonist effects at μ as well as κ receptors in the rat); 3) although icv $dynorphin_{17}$, the purported κ ligand, produces a dose-related hypothermia, the effect may be due to the peptide's rapid removal from the brain and stimulation of κ receptors elsewhere. $Dynorphin_{13}$, injected into POAH, produces only hyperthermia; 4)immunocytochemical and receptor autoradiographic studies indicate that the POAH has scattered prodynorphin fibers and appears to have many μ receptors but very few κ (M. Lewis, personal communication). Supported in part by Grant DA00376 from NIDA.

310.18 NALOXONE, NALTREXONE, AND BODY TEMPERATURE. R. Eikelboom. Dept. of Psychology, Queen's Univ., Kingston, Canada, K7L 3N6.

Naloxone causes a dose-related fall in body temperature (Eikelboom and Stewart, Life Sci., 28:1047, 1981). Naltrexone, a related compound, is more potent and longer lasting on a variety of narcotic antagonist measures (Blumberg and Dayton, Narcotic Antagonists, ed. M. Braude, p.33, 1974). Therefore these two compounds were compared for their effects on body temperature.

Seventy-four male Wistar rats (300 to 350 g) were habituated by repeated experience with all the procedures of the experiment. Rats were injected (IP) with saline or 1, 3, 10, 30 mg/kg Naloxone or Naltrexone (n=8, 2 saline groups n=5). Body temperature (rectal probe inserted 6cm) was measured -45, 0, 45, 90, 180, 270, 360, and 450 min after the injection, as well as 24 h later. There were no significant group differences at the -45 and 0 times but there was a significant dose related hypothermia at 45 and 90 min after the injection (maximum hypothermia was 0.6° C at 45 min). The two drugs were equipotent in terms of their hypothermic effects. At 90 min the effects were reduced for both drugs and by 180 min the groups no longer differed. At 270 and 360 min after the injection there was again a group difference with the naloxone and naltrexone animals exibiting a dose-related hyperthermia (increased temperature) relative to saline injected animals (maximum hyperthermia 0.5°C). At all later times the groups did not differ. Thus, it seems that for both of the body temperature effects the drugs seemed equipotent and their effects had similar time courses.

The effects of 30 mg/kg of naltrexone and naloxone on body temperature during the light and dark part of the cycle were investigated in 50 rats using similar procedures. As might be expected animals were always warmer during the dark. Again, both drugs induced equivalent hypothermic effects both in terms of maximum effects and time course. A comparison of the drug effect during the light and dark reveals that there were no circadian differences.

While both of the temperature effects of these drugs may not be pronounced they have been reported before with nalaxone so they appear real. More importantly it appears that these drugs are equipotent and have equivalent time course in their effects on body temperature. This is in marked contrast to the effects of these drug as narcotic antagonists. The temperature effects of these drugs may be mediated by different mechanisms than their antagonist effects.

310.19 VENTRAL TEGMENTAL MORPHINE INFUSIONS THROUGH VERTICLE BUT NOT ANGLED CANNULA HAVE AVERSIVE EFFECTS IN RATS. H.O. Pettit. Dept. of Psychology, Texas Christian University, Fort Worth, Texas 76129.

In a two part experiment infusions of morphine in the ventral tegmental area were paired with the taste of one of two novel flavored solutions. In the first experiment 19 male rats were given permanent verticle cannula implants aimed at the ventral tegmental area (VTA). Subsequently, the animals were conditioned to associate the effects of 1.0 µg/cannula injections of morphine sulfate or the 0.5 µl physiological saline vehicle with the taste of one of two novel solutions. Following conditioning, taste preferences for the two solutions were measured. Animals drank significantly less of a solution which had been paired with effects of morphine than of a solution which was paired with effects of the saline vehicle. Hence, rats developed conditioned taste aversions (CTAs) and demonstrated that morphine infusions through cannula aimed at the VTA can produce aversive effects. Experiment #2 was designed to examine dose response effects of morphine delivered through cannula implanted on a 20° angle to avoid the possible flow of morphine up the cannula to the periventricular grey region. Before conditioning trials the 40 experimental animals were additionally given a single injection of their respective morphine dose (0.00, 0.01, 0.1 or 1.0 µg/cannula) to attenuate the novelty of morphine effects and to reduce the ill effects of the lesions caused by the injections. The subjects in this experiment did not develop the CTAs observed in experiment #1. The results found in both experiments reveal that aversive effects of morphine injected into the VTA can occur, but they do not seem to be mediated by a central VTA component. Moreover, the present findings reveal that the aversive effects from VTA morphine applications can be attenuated with the use of procedural controls.

310.20 SINGLE UNIT ACTIVITY AND EFFECTS OF MORPHINE IN THE AMYGDALOID NUCLEAR COMPLEX OF THE RAT. W.W. Pugh, N.R. Walker*, M.W. Julian*, and C.G. Lineberry. North Carolina Foundation for Mental Health Research, Raleigh, NC 27611 and Medical Division, Burroughs Wellcome Co., Research Triangle Park, NC 27709

Morphine has been shown to inhibit or depress neuronal responses to noxious peripheral stimulation at spinal, medullary and thalamic levels. Thresholds of reflex tail movements and of vocalization following electrical stmulation to the tail are likewise elevated with systemic morphine administration. Tail flicks are regulated by spinal mechanisms while vocalizations are thought to be mediated at the supra-spinal level, and as such, may be part of the nocifensive or motivational response to pain. Intrathecal injections of morphine block both responses while morphine microinjected into the amygdala suppresses only the vocalization component of tail stimulation. Accordingly, spontaneous single unit activity in the amygdala was recorded extracellularly with glass micropipettes in urethane anesthetized rats. Thirty-four cells have been characterized physiologically with regard to mean firing rates, mean interspike intervals ± standard deviations, interspike interval histograms, and percent bursts and pauses. All cells reported here have been histologically confirmed to be within the amygdaloid nucleus complex by HRP marking of the recording site(s). Average firing patterns were slow (<1 Hz) and irregular. Several of these cells were tested for sensitivity to opiates by systemic administration of morphine (1-10 mg/kg) and/or naloxone (1-10 mg/kg). The majority of cells studied were inhibited by morphine, exhibiting a 50-90% decrease in spontaneous activity. Naloxone readily reversed these effects and typically elevated firing rates above baseline for a period of 30-40 minutes. Conversely, some neurons were moderately excited by morphine and greatly inhibited by naloxone while other cells did not respond to either. Thus, complex responses to morphine in a limbic structure (i.e., the amygdala) may participate in the affective component of nociception.

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311.1 ELECTROPHYSIOLOGICAL AND PHARMACOLOGICAL PROPERTIES OF MOUSE BRAIN DOPAMINE-CONTAINING VENTRAL TEGMENTAL AREA NEURONS RECORDED IN VIVO AND IN VITRO.

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The present studies examined the electrophysiological and pharmacological properties of dopamine-containing ventral tegmental area neurons in the mouse. These neurons were examined using extracellular single-unit recording techniques in both chloral hydrate anesthetized mice and in vitro mouse midbrain slices. In the in vivo preparation the ventral tegmental area neurons had long duration action potentials (2-5 ms), discharged at a rate of 1-9 spikes/s, and the cells discharge with either a decremental burst pattern or with a regular pattern. Systemic administration of the dopamine agonist, apomorphine, decreased the firing rate of these neurons, and the dopamine receptor blocker, haloperidol, reversed this effect. Similarly, systemic administration of the dopamine-releasing agent, D-amphetamine, suppressed the discharge rate of these neurons. This latter effect was blocked by pretreatment of the animals with α-methyl-p-tyrosine. When recorded in vitro from midbrain slices, ventral tegmental area neurons showed electrophysiological properties that were similar to those found in vivo, however, the neurons recorded in vitro fired at a significantly faster rate and the firing pattern in vitro tended to be more pacemaker-like, especially when recordings were made in an incubation medium which blocked synaptic transmission (e.g., low calcium/high magnesium). The activity of most of these neurons was suppressed by addition of apomorphine to the incubation medium, an effect that was reversed by haloperidol. Pretreatment with α-methyl-p-tyrosine produced no significant change in the discharge pattern or rate for cells recorded in vitro. These data indicate mouse ventral tegmental area dopamine neurons in vivo exhibit the same electrophysiological and pharmacological properties as rat and cat dopamine-containing neurons and that in vitro mouse ventral tegmental area neurons fire with pacemaker regularity in a low calcium/high magnesium medium. The in vitro preparation offers an approach to examining the fundamental properties of ventral tegmental area dopamine-containing neurons in the absence of afferent inputs.

311.2 PARTIAL AND FULL AUTORECEPTOR AGONIST EFFECTS OF DOPAMINOMIMETICS: ELECTROPHYSIOLOGICAL STUDIES. L.T. MELTZER AND C.CHRISTOFFERSEN* WARNER-LAMBERT/PARKE-DAVIS PHARMACEUTICAL RESEARCH, ANN ARBOR, MI.

Recently a number of compounds that are claimed to have selective actions on dopamine (DA) autoreceptors have been synthesized. In addition, it has become evident that the baseline firing rate of the DA neuron being recorded is an important determinant of the magnitude of the observed effect. We evaluated the effects of DA autoreceptor agonists on DA neurons of differing baseline rate. Cells that had a baseline firing rate of <4 Hz were termed slow firing; cells that had a baseline rate of >4 hz were termed fast firing. Experiments were conducted in chloral hydrate anesthetized male S-D rats. Using standard extracellular recording techniques, single DA neurons were identified by their waveform, firing pattern, and subsequent histological verification of recording site. Drugs were administered IP or IV. We first demonstrated that the population of DA neurons that were being recorded from were nigrostriatal neurons. DA neurons that had baseline firing rates up to 8 Hz were antidromically activated from stimulating electrodes in the caudate nucleus. The dose-response curve for inhibition of fast firing DA cells (mean rate = 6.2 Hz) by IV apomorphine (APO) was similar to that previously reported for nigrostriatal DA neurons ($ED_{50}$ = 8 ug/kg; 100% inhibition at 128 ug/kg). APO (1 mg/kg, IP) and (+)-3PPP (5 mg/kg, IP), completely inhibited slow and fast firing DA neurons. Similar to APO and (+)-3PPP, (-)-3PPP (5 mg/kg, IP), completely inhibited slow firing DA cells. In contrast to APO and (+)-3PPP, (-)-3PPP, at 20 mg/kg IP could only partially inhibit (60%) fast firing DA cells. Initial observations that (-)-3PPP produced a dose-dependent antagonism of the inhibitory effects of APO (1 mg/kg, IP) on fast firing DA neurons and that low doses of APO (0.1 mg/kg, IP) or (+)-3PPP (1.25 mg/kg IP), which produced a partial inhibition of fast DA neurons, did not antagonize the effects of APO (1 mg/kg, IP), suggested that the ability to antagonize the inhibitory effects of APO on fast firing DA neurons might provide a test for partial agonist activity. However, further testing revealed that there were some neurons that were partially inhibited by APO (0.5 mg/kg, IP) that could not be completely inhibited by a subsequent dose of APO (1 mg/kg, IP) . Thus, the ability to antagonize the effects of APO (1 mg/kg, IP) on fast firing DA neurons, does not represent an accurate test of partial autoreceptor agonist activity. This may be better reflected by the ability of the test compound, itself, to completely inhibit the activity of fast firing DA neurons. Thus, APO and (+)-3PPP act as full agonists and (-)-3PPP acts like a partial agonist at DA autoreceptors.

311.3 THE ACTION OF DOPAMINE ON THE AFTERHYPERPOLARIZATION RECORDED IN HIPPOCAMPAL PYRAMIDAL CELLS. R.C. Malenka and R.A. Nicoll. Dept.'s of Pharmacology & Physiology, U.C.S.F., San Francisco, CA. 94143

Dopamine (DA) has been reported to have long lasting effects on the calcium-activated potassium conductance ($G_{K(Ca)}$) underlying the slow afterhyperpolarization (AHP) which follows a train of action potentials. However both increases in the AHP, associated with a slow hyperpolarization, and decreases in the AHP, associated with a slow depolarization, have been reported. Using the rat hippocampal slice preparation we have examined the actions of DA on CA1 pyramidal cells paying particular attention to its effects on $G_{K(Ca)}$. Bath applied DA (1-100 μM) significantly reduced the AHP in a dose dependent fashion. This effect was completely reversed after 10-15 minutes of washing and with higher doses of DA was often associated with a small depolarization. The blockade of the AHP likely occurs at a step subsequent to the entry of Ca since Ca spikes recorded in 0.3 μM TTX and 5 mM TEA were not affected by DA. Neither the amount of current injected to elicit the AHP nor its initial amplitude had an effect on the reduction of the AHP by DA. Since DA's actions closely mimicked those of norepinephrine and DA has been shown to interact with β receptors, we examined the effect of β antagonists on DA's actions. At concentrations which have been shown not to block DA stimulated adenylate cyclase, both propranolol and timolol (5 μM) completely inhibited the reduction of the AHP by DA. This strongly suggests that this major action of DA in the hippocampus is due to its cross reactivity with β receptors.

To determine whether there might be some action of DA unrelated to adenylate cyclase stimulation (i.e. mediated by a D2, not a D1 receptor) we examined the actions of apomorphine, a D2 agonist, and those of DA in the presence of β antagonists. Bath application of these agents elicited no consistent change in any measurable electrophysiological parameter. Because DA may inhibit acetylcholine release in other neuronal systems we also looked at whether DA blocked the slow cholinergic EPSP which can be elicited in the hippocampus. Again, DA had no effect.

In some cells using either iontophoresis or puffer application of high concentrations of DA we elicited small (1-5 mV) hyperpolarizations. This action of DA was not antagonized by either chlorpromazine or haloperidol (1 μM) raising doubts that it is mediated by DA receptors. Since the size of these responses was small and their occurrence unpredictable, we have not pursued their pharmacological characterization.

In summary, we were unable to find any neuronal action of DA in the hippocampus that could be attributed unambiguously to its specific interaction with DA receptors. Instead we are forced to conclude that its major electrophysiological action (i.e. a blockage of the AHP) is due to cross reactivity with β adrenergic receptors. Supported by the Scottish Rite Schizophrenia Research Program, NMJ, USA and Grants NS07495 (to R.M.) and MH38256, MH00437 (to R.N.).

311.4 STUDIES ON THE MECHANISM OF DOPAMINE AUTORECEPTOR-INDUCED INHIBITION OF DOPAMINERGIC TRANSMISSION IN RABBIT STRIATUM.

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It is now well established that autoreceptors are present on dopamine cell bodies, dendrites and axonal terminals which act to decrease neuronal activity and neurotransmission in the mammalian nervous system. The purpose of the present study was to explore the mechanism of the inhibitory effect of dopamine autoreceptors on neurotransmission in the neostriatum of the rabbit. The hypothesis examined was that activation of autoreceptors inhibits the evoked release of dopamine by limiting the availability of calcium necessary for secretion coupling. Autoreceptor function was assessed by examining the effect of dopamine agonists on the release of dopamine produced by field stimulation of superfused slices. Similar experiments were carried out following perturbations that were shown to enhance the release of dopamine presumably by increasing the entry of calcium into nerve terminals. Pertubations included raising the extracellular $Ca^{2+}$ concentration in the superfusion buffer, administration of aminopyridine or the administration of $Ca^{2+}$ ionophore A23187.

Rabbits were decapitated, the brain quickly removed and the striatum dissected. Sagittal striatal slices (0.3 mm) were prepared with a MacIlwain tissue chopper. Following a preincubation, slices were incubated in a physiological buffer containing $^3$H-dopamine ($10^{-7}$ M) and then placed in individual superfusion chambers. Superfusate effluents were continuously collected by a fraction collector. Apomorphine was shown to produce a concentration dependent decrease in the field stimulation induced release of $^3$H-dopamine. The release of $^3$H-dopamine was measured in the absence ($S_1$) or presence ($S_2$) of apomorphine and the $S_2/S_1$ ratio calculated. $S_2/S_1$ ratios were 0.99 ± .2, 0.67 ± .17 and 0.53 ± .08 for control and $10^{-7}$ or $10^{-6}$ on apomorphine, respectively. Although lower concentrations were without effect, aminopyridine ($10^{-4}$ M) produced a marked facilitation of the field stimulation induced release of $^3$H-dopamine resulting in a $S_2/S_1$ ratio of 2.83 ± .59. Apomorphine ($10^{-6}$ M) was still able to decrease the evoked release of $^3$H-dopamine in the presence of aminopyridine with a $S_2/S_1$ ratio of 0.80 ± .3.

The results to date suggest that activation of dopamine autoreceptors does not inhibit the release of dopamine by preventing the entry of extracellular calcium across the nerve terminals.

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311.5 NEUROPHARMACOLOGY OF MESENCEPHALIC DOPAMINERGIC NEURONS IN VITRO. M. S. Brodie and T. V. Dunwiddie, Dept. Pharmacology, University of Colorado Health Sciences Ctr. and Veterans Administration Medical Research Service, Denver, CO 80262.

Mesencephalic dopaminergic neurons are found primarily in the ventral tegmental area of Tsai (VTA) and in the substantia nigra (SN). Because these areas appear to mediate different types of behaviors, interest has developed in agents that may selectively affect the activity of these subgroups of dopaminergic neurons. In order to study the pharmacology of neurons in these areas in a quantitative fashion, an in vitro preparation containing both the VTA and SN has been developed.

Sprague-Dawley rats were sacrificed, the brain quickly removed, trimmed, affixed to a vibratome chuck, and plunged into artificial cerebrospinal fluid (aCSF) kept at 4°C. Slices (300 um) were cut in the coronal plane in such a fashion that both the VTA and SN were included in a single slice. The slices were transferred to the recording chamber, which was maintained at 35.5°C, and were allowed to equilibrate for one hour before recording was begun.

Intra- and extracellularly recorded action potentials of VTA neurons were similar to those recorded in intact anesthetized rats. Long duration action potentials (2.4-4 msec) were observed with an inflection on the rising phase corresponding to what has been considered to be the initial segment spike. The firing rate of these neurons was 1-3 Hz. The effects of various agents were tested on spontaneous firing rate of VTA neurons. Dopamine (10-100 uM) inhibited the firing rate of all VTA neurons (n=12) exhibiting the characteristics indicated above (type I neurons). Inhibition was also produced by dopamine agonists such as apomorphine, and could be reversed with fluphenazine, a specific dopamine antagonist. A subpopulation of non-dopaminergic neurons in the VTA (type II neurons) that have a shorter duration action potential and faster firing rate, did not respond to dopamine.

Substantia nigra neurons were more sensitive than the putative dopamine-containing type I neurons of the VTA to the inhibitory effects of dopamine. The firing of a majority of SN neurons was completely inhibited by 100 uM dopamine, whereas in the VTA this concentration of dopamine reduced the firing rate but did not produce a complete cessation of activity. These results suggest that there may be important differences in the pharmacology of dopaminergic neurons located in these two regions, and that certain drugs may have a relatively selective action upon dopaminergic neurons in these two groups.

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311.6 CAFFEINE AND MESOLIMBIC DOPAMINE NEURON ACTIVITY: ANTAGONISM OF CAFFEINE BY BOTH DIAZEPAM AND HALOPERIDOL. Gene R. Stoner*, Sidney Werkman*, Lana R. Skirboll, and Daniel W. Hommer. Clinical Neuroscience Branch, NIMH, Bethesda, MD 20205.

Caffeine is probably the most widely used psychotropic drug in the world today and its psychostimulant effects are well known. Although caffeine has recently been demonstrated to potently antagonize the effects of adenosine, it is not clear that the psychostimulant actions of caffeine are exclusively mediated through adenosine system. Since catecholamines, particularly dopamine (DA), have been implicated in the actions of other stimulant drugs and since caffeine has been demonstrated to increase the release of catecholamines both in peripheral tissue and in brain synaptosomes, we examined the effects of intravenously administered caffeine on the activity mesencephalic dopamine (DA) neurons in the rat ventral tegmental area (VTA).

Male Sprague-Dawley rats were anesthetized with chloral hydrate; placed in a stereotaxic apparatus and a burr hole drilled over the VTA. Standard extracellular single unit recording techniques were employed. Once a putitative VTA unit was encountered its identity as a DA neuron was confirmed using previously established criteria. After a five minute period of baseline recording, caffeine in doses of 5, 5, 10 and 20 mg/kg was administered intravenously (n=13). Caffeine produced a significant, dose-dependent decrease in the activity of VTA-DA neurons (ANOVA with repeated measures on dose, $F(1, 24) = 16.1$, $p < 0.03$). Following a cumulative dose of 20 mg/kg of caffeine the firing rate of VTA-DA neurons was 60 ± 8% of baseline. After 40 mg/kg the firing rate decreased further to 46 ± 8% of baseline. Diazepam, 0.5 mg/kg, almost completely blocked the inhibitory effects of caffeine (n=13). Haloperidol 0.5 mg/kg also completely blocked the inhibitory effects of caffeine on VTA neurons (n=8).

Our results suggest that caffeine's psychostimulant effects may in part be mediated by an action on DA systems. Since caffeine's effects can be blocked by haloperidol, caffeine may cause increased release of DA in forebrain regions which in turn leads to a feedback inhibition of DA neuron activity. It is also possible that caffeine could cause release of dendritic DA which could locally inhibit DA neurons. Thus, the actions of caffeine on mesolimbic DA systems may be similar to the actions of amphetamine since amphetamine decreases DA neuron activity by DA release and activation of an inhibitory feedback pathway (Bunney and Aghajanian in The Basal Ganglia, Raven Press, 249-267, 1976).

311.7 SUPERSENSITIVITY OF DOPAMINERGIC NEURONS TO APOMORPHINE IN RATS FOLLOWING CHRONIC HALOPERIDOL. G.D.Vogelsang and M.F.Piercey, CNS Research, The Upjohn Company, Kalamazoo, MI 49001.

Although acute neuroleptic treatment results in an effective blockade of post-synaptic dopamine (DA) receptors, chronic treatment is required for effective antipsychotic therapy. The clinical ineffectiveness of acute treatment may be due to increased firing rates of DA neurons observed following acute treatments. Recent experiments focusing on chronic neuroleptic treatment have demonstrated depression in numbers of spontaneously active DA neurons and enhanced DA autoreceptor sensitivity at nerve terminals. The present study further investigates these effects by examining the effects of chronic neuroleptic treatment on cell body autoreceptor sensitivity, indicated by dopamine agonist-induced depressions in DA neuron firing rates.

Microelectrodes filled with 2M NaCl saturated with 2% fast green were stereotaxically placed into the substantia nigra pars compacta of chloral hydrate-anesthetized rats. DA neurons were identified by large positive-negative spikes > 2.5 msec duration and histological localization of iontophoresed dye spots. The average effective dose of i.v. apomorphine (APO) for achieving 50% inhibition (ED50) of the spontaneous discharge of DA neurons in control rats was 9.6±3.2 ug/kg. The APO ED50 for rats receiving chronic haloperidol (1.0 mg/kg/day i.p. for 28 days followed by 3-day washout) was 3.4±0.8 ug/kg ($p<0.001$, t-test). Firing rates of DA neurons were unchanged. It is concluded that chronic haloperidol makes DA cell body autoreceptors supersensitive to agonist stimulation, an action which could contribute to the antipsychotic efficacy of chronic treatments.

311.8 CHARACTERIZATION OF PUTATIVE DOPAMINERGIC (DA) NEURONAL FIRING IN THE VENTRAL TEGMENTAL AREA (A10) OF UNRESTRAINED RATS. A.S. Freeman and B.S. Bunney, Depts. of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT

We have reported that putative substantia nigra zona compacta (A9) DA cells of unrestrained rats fire in a range of rates and patterns similar to those of conclusively identified DA neurons. In the present study, the firing properties of putative A10 DA cells in freely moving rats were investigated.

A steel well was cemented to the skull overlying the A9 or A10 region of male rats and an injection cannula was implanted into a jugular vein. Prior to recording sessions, a microdrive containing a glass-coated tungsten electrode was inserted into the implanted well. Putative DA cells (n=39) were readily distinguished on the basis of waveform, firing rate (5.0±1.3 spikes/s, $\bar{x}$±SD) and pattern. Most cells (90%) fired a portion (36±21%) of their spikes in bursts which consisted of up to 18 spikes ($\bar{x}$=2.9 spikes/burst).

Previous intracellular studies in gallamine-paralyzed and chloral hydrate-anesthetized (immobilized) rats have shown electrical coupling to occur between A9 DA cells (Grace and Bunney, Neurosci. 10: 333, 1983) and we have observed, extracellularly, a higher incidence of apparent A9 DA neuronal coupling in unrestrained vs. immobilized rats (Freeman et al., Life Sci. 36: 1983, 1985). Ten pairs of A10 cells (26%) in the current sample were also observed to interact in this manner. These findings strongly suggest that synchronization of midbrain DA neuronal firing in the behaving rat is an important means by which these neurons influence their target cells.

Presumed DA neuronal discharge was unrelated to gross motor behavior. However, small movements of the snout and vibrissae (as in sniffing) were often associated with prominent burst firing. Mechanical stimulation of the vibrissae produced a similar change in pattern. Exposure to light flashes (1-3 Hz) did not alter neuronal firing.

In preliminary pharmacological experiments, we have tested the effects of CCK-8 (a substance co-localized with DA in a subpopulation of midbrain cells) on A10 DA neurons. CCK-8 (1-16 μg/kg) produced inconsistent effects on firing. CCK-8 also produced variable effects on firing patterns which were not always related to changes in rate. Preliminary data suggest that CCK-8 enhances the inhibitory potency of apomorphine on A10 DA cell firing. In A9, CCK-8 either stimulated (stimulation block by progluminde, 200 μg/kg) or had no effect on firing rate. Each effect of CCK-8 generally lasted less than 2 min. These effects of CCK-8 are similar to those obtained in immobilized rats. Supported by MH-08987, MH-25642, MH-28849 and the State of Connecticut.

311.9 IN SITU INTRACELLULAR RECORDING FROM NUCLEUS ACCUMBENS NEURONS: I. SPONTANEOUS RHYTHMIC OSCILLATION OF RESTING MEMBRANE POTENTIAL. C.Y. Yim and G.J. Mogenson. Dept. of Physiology, University of Western Ontario, London, Ontario, Canada.

Interest in the unique anatomical and functional association of the nucleus accumbens (NAcc) with both limbic and presumed motor nuclei of the brain prompted a number of extracellular single unit recording experiments to investigate the input-output relationship as well as the effect of dopamine on cellular activity in this nucleus (Yim and Mogenson, Brain Res., 239:405, 1982). In order to further study the electrophysiological characteristics of neurons in the accumbens and the cellular action of dopamine, intracellular recordings were obtained from accumbens neurons in the urethane anesthetized (1.5 g/kg) rat in situ. Recordings were obtained using K acetate (2 M) or K citrate (1.5 M) filled glass micropipettes with impedance of 60-100 M ohm. Only recordings from neurons having resting membrane potentials of >45 mV and action potentials of >50 mV were analysed. Input resistance of accumbens neurons measured with small hyperpolarizing pulses (0.1 to 0.5 nA, 30 ms) was found to be 12-30 M ohm, similar to those reported in earlier studies with striatal slices (Chang and Kitai, Soc. Neurosci. Abstr., 10:703, 1984). An unexpected observation from this in situ preparation was that 65% of the accumbens neurons (n = 54) displayed spontaneous large amplitude rhythmic oscillations of the resting membrane potential. The frequency of the oscillations was from 0.6 to 0.9 Hz and the amplitude ranged from 5 to 20 mV. In some neurons, action potentials occurred at the peaks of the oscillation resulting in the characteristic bursting firing pattern observed in extracellular unit recordings. No comparable EEG, however, could be recorded from the extracellular space. Stimulation of the ventral tegmental area (VTA) with trains of 10 pulses (400 µA to 1 mA) at 10 Hz produced consistently 3 to 10 mV depolarization of the membrane potential and elimination of the spontaneous oscillations. The induced depolarization was accompanied by an average of 20% increase in membrane conductance. The effects of VTA stimulation lasted up to several minutes after cessation of the stimulation. Oscillations were not observed in presumed glia cells and their resting membrane potential was not changed by VTA stimulation either. These empirical findings provide the basis of further experiments in progress.

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311.10 IN SITU INTRACELLULAR RECORDING FROM NUCLEUS ACCUMBENS NEURONS: II. VENTRAL TEGMENTAL AREA STIMULATION ATTENUATES EVOKED EPSP FROM AMYGDALA STIMULATION. G.J. Mogenson and C.Y. Yim (SPON: J.J. Seguin). Dept. of Physiology, University of Western Ontario, London, Canada.

Previous experiments using extracellular single unit recording techniques showed that nucleus accumbens (NAcc) neurons are strongly excited by stimulation of the amygdala (AMY) and the hippocampus, and that the excitatory response is preferentially attenuated by stimulation of the ventral tegmental area (VTA), site of the A10 dopamine (DA) cell bodies. These and other results led to the suggestion that DA may act presynaptically to modulate synaptic transmission in the ventral striatum. This possibility was investigated further in the present study. Intracellular recordings were made from NAcc neurons of urethane anesthetized rats in situ using K acetate or K citrate filled micropipettes. Stimulation of the basolateral nucleus of the AMY (400 µA to 1 mA) elicited a depolarizing-PSP (dPSP) - hyperpolarizing-PSP (hPSP) sequence in 52 of 54 (96%) NAcc cells sampled. The amplitude of the dPSP varied between cells and ranged from 6 to >30 mV and was continuously monitored by a peak amplitude detector. Stimulation of the VTA (400 to 800 µA) in trains of 10 pulses at 10 Hz consistently depolarized the resting membrane potential and attenuated the amplitude of the dPSP. Although amplitude of PSPs usually varies with membrane potential, the attenuation of the dPSP by VTA stimulation did not appear to be entirely due to the concurrent membrane depolarization. This is suggested because similar depolarization of the membrane potential by injection of a small depolarizing current through the recording electrode did not produce an equivalent attenuation. Moreover, attenuation of the dPSP as well as depolarization of the membrane potential persisted in some cases for several minutes after termination of VTA stimulations but the time course of recovery differed between the two responses. The late hPSP was not consistently affected by the VTA stimulation in contrast to the effect on the dPSP, and in addition, a greater attenuation of the dPSP was observed when the recording was obtained using K citrate electrodes (-42%) compared to those obtained using K acetate electrodes (-33%). Taken together, these results are interpreted to indicate that AMY stimulation produces an EPSP-IPSP sequence in NAcc neurons. Partial reversal of the hyperpolarizing IPSP by acetate augments the EPSP and produces the dPSP-hPSP sequence observed. VTA stimulation produces membrane depolarization and preferential attenuation of the EPSP probably by different mechanisms, the latter involving a presynaptic mechanism in view of its selectivity. Both effects are likely due to DA released in the NAcc based on earlier observations from extracellular single unit recording experiments.

(Supported by MRC of Canada).

311.11 VALIDATION OF THE ACCURACY OF VOLTAMMETRIC MEASUREMENT OF BIOGENIC AMINE CONCENTRATIONS IN VIVO. J. Schenk*, A. Hansen, A. Knorr*, W. Stewart, and B. Bunney (SPON: L. Kitahata), Depts. Psychiatry, Pharmacology, Neuroanatomy and Neurosurgery, Yale Univ. Sch. of Med. New Haven, CT.

In vivo voltammetric measurements (IVVM's) have been shown to be useful for measuring oxidizable molecules in the extracellular fluid (ECF) of the brain. The compounds thought to be detected are the biogenic amines and their metabolites, ascorbic acid, and uric acid. Prior to experimentation, a carbon sensing electrode (dia. 8-40 um) is calibrated by applying a potential sufficient to oxidize the species of interest and measuring the resulting oxidation (faradaic) current over a range of concentrations (C). The slope of the signal vs. C line is the electrode calibration factor. Then the electrode is implanted into the brain and IVVM's are begun. Signals can be expressed in terms of C based on the in vitro calibration factor. Often the calibration serves as a normalization procedure for comparing responses obtained at multiple electrodes or between groups of experiments. However, the ultimate goal is to measure signals in terms of "absolute" C changes. These values are needed for making correlations with receptor affinities, uptake carrier affinities, and enzyme Km's. However, the accuracy of in vivo C measurement is unknown. To assess the accuracy of IVVM's of C based on in vitro calibration one needs a "standa*d" compound that can be introduced into the brain quantitatively, that diffuses through the ECF in a defined way, and can be measured in vivo in the ECF by an alternate analytical technique. Our approach to develop this "standard" is to fabricate an Aliquot 336-based ion selective electrode (ISE) (dia. 1-2 um) for ferrocyanide (FeCN6) ion to use to independently measure an ion that can also be detected faradaically. This ion is a good probe since the FeCN6 complex is stable at neutral pH (formation constant = 10^24), its faradaic electrode reaction is known, and a diffusion coefficient for it in the ECF has been reported (Rice et. al. Neurosci. in press 1985) . In our poster, we describe the fabrication and properties of the FeCN6 ISE and illustrate its usefulness for measuring FeCN6 ion in the ECF. Detailed analysis comparing diffusion profiles obtained using ISE's and IVVM's will be presented. Preliminary results suggest that short duration IVVM's (tens of ms.) based on in vitro calibrations gives C values that match those obtained with ISE's. However, relatively long IVVM's (> 1 sec.) result in deviations from ISE values suggesting that the best in vivo voltammetric techniques for C measurements are those utilizing electrolysis times of one sec. or less. Support from MH-28849, MH-25642, NIMH Postdoc. Fellows. 1 F32 MH09081 (J.S.), NSF Predoct. Fellow (A.K.), Weimanns Legat, NINCDS NS 16933 (A.H., W.S.) and the State of CT. is gratefully acknowledged.

311.12 THE EFFECT OF KNOWN CONCENTRATIONS OF DOPAMINE ON DOPAMINE CELL FIRING RATES IN MIDBRAIN TISSUE SLICES. N.L. Silva, J.O. Schenk* and B.S. Bunney, Depts. of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06510

Previous anatomical studies have demonstrated the existence of dopaminergic (DA) neurons within the pars compacta of the substantia nigra. These cells are readily identifiable by their electrophysiological characteristics and pharmacological responsiveness. DA neurons are typically inhibited by the administration of DA or DA agonists, presumably through an action on cell body or dendritic autoreceptors. Recently, in vitro studies have shown that neurons possessing this characteristic waveform were inhibited by DA. The purpose of this study was to investigate in detail the response of midbrain DA cells to known DA concentrations by analyzing the concentration dependent reduction of DA cell firing rate in the context of equilibrium models.

Rat midbrain tissue slices (300-400 µm) were placed in a humidified chamber which was constantly perfused with an oxygenated nutrient medium (pH 7.4, 300 mOsm/kg, 10 mM glucose) and maintained at 36°C. The perfusate could be rapidly switched between normal and experimental medium using three-way stopcocks. Each neuron encountered was recorded for 3 to 5 minutes prior to the introduction of DA containing medium (0.5-500 µM). DA concentrations in the perfusate were confirmed by high pressure liquid chromatography with electrochemical detection. DA concentrations in the extracellular fluid of the slice were monitored using carbon fiber voltammetric electrodes (dia. 40 µm) implanted 150 µm into the slice. After a maximal change in firing rate was observed, the perfusion was switched back to the normal nutrient medium. Baseline firing rate was always re-established before any additional DA concentrations were tested.

Preliminary studies show that DA interaction at DA-receptive sites in the midbrian slice preparation results in a suppression of firing rate. In these experiments, 2 subpopulations of DA cells were defined according to their response to DA. One group has an apparent DA DA-receptive site dissociation constant (K') of approximately 10 µM while the second group has a K' value of approximately 160 µM. Both groups follow a simple equilibrium relationship having a stoichiometric relationship of 1:1. It is hoped that development of this paradigm will provide a more precise basis for the study of the action of DA, modulatory substances and DA agents on DA neuronal system functioning.

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311.13 ELECTROPHYSIOLOGICAL AND PHARMACOLOGICAL EVIDENCE FOR THE EXISTENCE OF TWO TYPES OF NIGROSTRIATAL DOPAMINERGIC NEURONS. D.C. German and P.D. Shepard. Depts. of Physiol. and Psychiat., Univ. of Texas Health Sci. Cntr., Dallas, TX. 75235.

Substantia nigra (SN) dopamine (DA)-containing neurons have been reported to exhibit two predominent patterns of firing. Previous studies in which DA cells were systematically recorded throughout the rostral-caudal extent of the SN have revealed a tendency for cells which fire with an irregular pattern to be located in the rostral half of the nucleus whereas cells which fire with a bursting pattern reside primarily in the caudal half of the nucleus. Comparison of the sensitivity of Bursting and Irregular firing cells to an autoreceptor-selective dose of apomorphine has revealed that Irregular cells were significantly more sensitive to the rate-decreasing effects of the drug than Bursting cells (Shepard & German, Neurosci. Abstr., 8:261.1, 1982; Neurosci. Abstr., 10:280.7, 1984).

The purpose of the present experiment was to use single cell recording and antidromic activation techniques to determine whether these two cell types (a) project to similar neuroanatomical regions, and (b) exhibit similar sensitivity to the rate-depressing effects of the indirect acting DA agonist, d-amphetamine (d-AMP). Male albino rats (250-300 g) were anesthetized with chloral hydrate (400 mg/kg, i.p.) and the femoral vein was cannulated for subsequent drug administration. Standard electrophysiological recording techniques were employed. Only one cell was studied per rat. Cells were classified into groups based upon their interspike interval histogram shape; Irregular cells exhibited a normal-like ISI distribution, whereas Bursting cells exhibited a positively skewed ISI distribution (1000 spikes/histogram). We found that both cell types project to the caudate-putamen, however, the estimated conducting velocities were significantly different (Irregular = 0.32 m/s, Bursting = 0.60 m/s, $p < .001$). We also found that Irregular cells were significantly less sensitive to low doses of d-AMP (0.5 mg/kg, i.v.) but significantly more sensitive to higher doses (1.5-4.0 mg/kg) than Bursting cells. These data indicate that nigral DA neurons which exhibit different firing patterns are characterized by different axonal properties and sensitivities to direct and indirect acting DA agonists. The Bursting neurons are faster conducting, less sensitive to autoreceptor stimulation but more sensitive to the effects of low doses of d-AMP than cells which exhibit an Irregular firing pattern. These two cell types may coincide with the two different cell body morphologies found in the SN (Fallon et al., Neurosci. Lett., 7:157, 1978) and the two predominant types of DA nerve terminals in the striatum (Kaiya & Namba, Neurosci. Lett., 25:251, 1981). Research supported by grant MH-30546.

311.14 **NEURONS LOCATED IN THE VENTRAL GLOBUS PALLIDUS (SUBSTANTIA INNOMINATA) DEMONSTRATE AN APOMORPHINE-INDUCED INCREASE IN ACTIVITY WHICH IS REVERSED BY HALOPERIDOL.** **T.C. Napier, P.E. Simson* and G.R. Breese. Univ. of North Carolina Sch. of Med., Chapel Hill, NC 27514 and Duke Univ. Sch. of Med. Durham, NC 27709.**

**The ventral aspect of the forebrain, traditionally denoted as substantia innominata, is now being characterized as comprising distinct groups of cells. Heimer and Wilson (Golgi Centennial Symposium, Proceedings, M. Santini (ed) Raven Press, NY, 1975, pp. 177-193) anatomically described for the rat an area immediately ventral to the anterior commissure and rostrally situated at the level of the external segment of the globus pallidus. This region, designated as the ventral pallidum, receives afferents ventral striatal regions (as well as the nucleus accumbens) and perhaps serves in a circuit which is parallel to "dorsal" striatum → "dorsal" globus pallidus projections. Since the globus pallidus is known to be influenced by systemically administered dopamine agonists (Bergstrom, Bromley and Walters, Brain Res. 238:266, 1982; Napier, Simson and Breese, Fed. Proc. 5:1387, 1985) this study was undertaken to determine if neurons of the ventral pallidum are also affected by dopaminergic drugs. Using standard electrophysiological techniques, extracellularly recorded single units were obtained from chloral hydrate anesthetized male Sprague Dawley rats. Following 5-10 min of pretreatment monitoring, apomorphine was administered via a cannulated lateral tail vein. The doses used were 1 ug to 1 mg/kg, injected every two min so that each injection doubled the previous dose. Haloperidol (0.1 to 1 mg/kg i.v.) was administered at the conclusion of apomorphine treatments. Using this protocol, the majority of ventral pallidal units responded to apomorphine with an increase in firing rates generally 100% above pretreatment levels. This increase was antagonized by haloperidol. Some cells were not affected by the doses of apomorphine used in these experiments. These studies indicate that, like the dorsal globus pallidus, neuronal activity of the ventral pallidum/substantia innominata are dramatically altered by dopaminergic drugs. (Work supported by F32-NS07247, FT32-MH15177, HD-03110 and MH-33127).**

311.15 Depolarization Inactivation of Nigral Dopamine Neurons by Repeated Haloperidol Administration: Analysis by in vivo Intracellular Recording. B.S. Bunney & A.A. Grace, Departments of Psychiat. & Pharmacol., Yale Univ. Sch. Med., New Haven, CT 06510 and Depts. of Psychol. and Psychiat., Univ. Pittsburgh, Pittsburgh, PA 15260.

Acute haloperidol treatment in rats is known to increase the firing rate of nigral dopamine (DA) neurons. However, in 1978 we reported that repeated treatment of rats with haloperidol (0.5 mg/kg for 21 days) resulted in a dramatic decrease in the number of spontaneously firing DA neurons in the substantia nigra. Since these nonfiring cells could not be activated by microiontophoretic application of excitatory substances (e.g., glutamate) but could be activated by inhibitory agents (e.g., GABA), we hypothesized that the DA neurons were in a state of depolarization inactivation or depolarization block. However, by using extracellular recording techniques, we were only capable of providing indirect evidence that the DA cells were depolarized to the extent that they were incapable of generating spontaneous action potentials.

More recently, we have attempted to obtain direct evidence for the depolarization block model of inactivation by recording intracellularly from identified DA neurons in vivo. In control rats, DA neurons have an average resting membrane potential (RMP) of about 55 ± 3 mV, and were either firing spontaneously or would discharge in response to depolarization of the cell membrane. However, following repeated haloperidol administration the impaled DA cells had significantly more depolarized RMPs (41 ± 6 mV) and were incapable of firing action potentials either spontaneously or in response to membrane depolarization. Although it is difficult to rule out injury as a possible source of this depolarized state, we believe that this reflects the actual membrane potential of the DA cell after repeated haloperidol administration since: 1) almost every DA cell impaled had a resting potential near this level, 2) the RMP was stable over time, 3) the input resistances of the cells were not significantly different from controls (29 ± 7 megohms, as compared to an input resistance of 31 ± 7 megohms in untreated rats), whereas injured cells typically have input resistances of <10 megohms, and 4) the depolarization inactivated state could be reversed by hyperpolarizing current injection or by pharmacological means 5 minutes or longer after impalement. Thus, after impalement, hyperpolarization of the cell by current injection restored spontaneous firing of the DA cell. Furthermore, systemic administration of apomorphine caused DA cells in depolarization inactivation to repolarize in a dose-dependent stepwise manner which led to a resumption of spontaneous activity. In summary, repeated haloperidol treatment induced a state of depolarization inactivation in DA neurons by depolarizing the membrane potential to the extent that the spike generating mechanism was inactivated. (Supported by USPHS MH28849, MH25642, and the State of Connecticut)

311.16 POSSIBLE FACTORS DETERMINING THE PROPERTY OF ANTIDROMIC DISCHARGES OF DOPAMINERGIC A10 NEURONS DURING REPETITIVE ACTIVATION. K.Watabe, T.Shinba*, R.Sugita*, and T.Satoh*. Dept. of Physiol., Aichi-Gakuin Univ. Dent. Sch., Nagoya, 464 Japan.

In the noradrenergic(NA) A6 and serotonergic(5-HT) raphe neurons, the latency of antidromic discharges is gradually prolonged to reach an asymptote during repetitive activation. We have suggested that although the way of latency prolongation is apparently similar in the two monoaminergic sytems, the mechanism behind those phenomena may not be identical. The present study aimed at investigating whether latency prolongation upon repetitive stimulation can occur also in dopaminergic(DA) neurons, and if it is the case, what kind of mechanism is operating in these neurons. For this purpose unit activity of meso-accumbens(Acc) A10 neurons was extracellularly recorded in normal and chemically treated rats (haloperidol(HAL) or picrotoxin(PTX) i.p., kainic acid(KA) locally into the Acc, or HAL combined with KA(KA+HAL)). Gradual latency increase during 10Hz stimulation, similar to that observed in the NA or 5-HT neurons, was found in a great majority of neurons of normal and chemically treated rats. The increase in latency, as expressed in % of the initial latency, was greatest in the normal (mean, 11.3%), smallest in the KA+HAL(6.2%), and intermediate in the KA(8.6%), PTX(9.7%) and HAL(10.5%). Some neurons in the KA+HAL rats showed, in the course of latency prolongation, "latency jump" followed by reprolongation of latency. All the neurons showing latency prolongation were apparently dopaminergic, as judged from their slow spontaneous firing and wide duration of spikes. In the antidromic responses during 10Hz stimulation in normal rats, 77% of the initial segment(IS) spikes were not followed by the somadendritic(SD) spikes. In contast, in all the chemically treated rats, the frequency to obtain suppression of antidromic SD spikes was extremely or moderately low; 2.7%, 9.5%, 38% and 55% in the KA+HAL, KA, PTX and HAL, respectively. These results suggest that both axonal and somatic mechanisms are involved in the latency prolongation during repetitive activation. More frequent suppression of antidromic SD spikes and more marked latency prolongation of IS spikes in normal rats may be explained by activation of the afferents from the Acc, which is partly GABAergic, and also by self-regulatory mechanism in A10 DA neurons.

311.17 ELECTROPHYSIOLOGICAL AND PHARMACOLOGICAL PROPERTIES OF PRESUMED DOPAMINE-CONTAINING NEURONS IN THE SUPRAMAMMILLARY REGION OF THE RAT. P.D. Shepard and D.C. German, Depts. of Physiol. and Psychiat., U. of Texas Health Sci. Cntr., Dallas, TX. 75235.

Previous neuroanatomical studies have identified a small collection of catecholamine-containing cells within the supramammillary (SUM) complex (Fuxe, et al., In: Metabolism of Amines in the Brain. G. Hooper, (Ed), pp. 10-22, 1969). These cells appear to be dopaminergic (DA) since they contain tyrosine hydroxylase (TH), but not dopamine-beta-hydroxylase (Swanson, et al., J. Comp. Neurol., 163:467-506, 1975). SUM neurons have been shown to project to a number of limbic areas including the lateral septum (LS) and anterior limbic cortex (ALC). However, recent studies have shown that while approximately half of the LS-projecting cells contain TH, none of the ALC-projecting neurons appear to be dopaminergic (Swanson, Brain Res. Bull., 9:321-353, 1982). The purpose of the present study was to examine the electrophysiological and pharmacological properties of antidromically identified neurons within the SUM complex. In addition, the responsiveness of these cells to DA agonists was assessed to determine whether putative DA-containing neurons possessed rate-modulating autoreceptors. Action potentials from spontaneously active SUM neurons were biphasic and of moderately long duration (2.0-3.4 msec). Cells frequently exhibited slow firing rates (1.35 ± 0.2 impulses/sec) and erratic discharge patterns. Conduction velocities obtained from LS-projecting SUM neurons were not significantly different from ALC-projecting cells. Iontophoretically applied DA was without effect on spontaneously active SUM neurons. Similarly, low doses of apomorphine (5.0 ug/kg, i.v.) produced a significant decrease in the firing rates of only 3 of 11 cells tested. In contrast, D-amphetamine (0.5-2.0 mg/kg, i.v.) produced a significant decrease in 83% of the cells tested. In addition to spontaneously active neurons, several silent cells were encountered which were antidromically activated from the LS. These cells appeared to be tonically hyperpolarized as iontophoretic application of glutamate, but not GABA, was effective in causing the cells to discharge. Spike durations and estimated conduction velocities associated with silent SUM neurons were similar to those obtained from spontaneously active SUM cells. However, in contrast to the latter group, 100% of the silent cells sampled, induced to fire with glutamate, exhibited a statistically significant decrease in firing rate in response to iontophoretic DA. These data indicate that both NON-DA and DA containing cells exist in the SUM and that the DA-containing cells are normally silent in the anesthetized preparation. Research supported by MH-30546, Bristol-Myers Co. and The Upjohn Co.

## CATECHOLAMINES: ELECTROPHYSIOLOGY

312.1 A FUNCTIONAL AND MORPHOLOGICAL STUDY OF NORADRENERGIC INNERVATION OF THE SUPRAOPTIC NUCLEUS IN THE AGING RAT. W.F. Silverman, C.D. Sladek, and J.R. Sladek, Jr. Depts. of Anatomy and Neurology, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642.

The hypothalamic supraoptic nucleus (SON) of the rat exhibits a dense plexus of noradrenergic (NA) fibers and varicosities originating in brain-stem reticular formation. This plexus is most concentrated in ventral, vasopressin-rich portions of SON, though in the vasopressin-deficient Brattleboro mutant, and in aged rats, an apparent reorganization to more dorsal, oxytocin-rich areas is observed (Sladek et al., Peptides 1:141, 1981). An experiment to correlate this morphologically demonstrable change in the topographic localization of NA within the SON with the vasopressin responsiveness to an osmotic challenge was performed. Aged (24 month) and young (3 month) Fischer 344 male rats were deprived of water for 72 hours prior to sacrifice. Following decapitation, the brains were frozen and 16 um sections were cut on a cryostat. Tissue was treated for demonstration of glyoxylic acid-induced monoamine fluorescence and examined in a fluorescence microscope. Serum and neurohypophysis were also collected from each subject for future correlative assessment of vasopressin levels with respect to the visualized catecholamine input to the SON.

A dense pattern of fluorescent noradrenaline terminals was concentrated principally in ventral portions of SON among vasopressinergic neurons in young subjects. Challenged subjects did not appear to differ from unchallenged controls with respect to the NA innervation pattern. A less dense fluorescence pattern was observed at all levels in the SON of aged subjects in agreement with previous results obtained by this laboratory. Occasional cells in dorsal SON were observed which exhibited the apparent hyperinnervation by catecholamine terminals characteristic of this nucleus in the aging rat. These results confirm data from previous studies of changes in NA innervation of VP neurons in SON in aging. Previous studies in our laboratory have demonstrated an attenuated VP response to osmotic challenge in aged rats (Sladek et al., Neurobiol. Aging 2:293, 1981). It is conjectured that the reduction in CA in the SON during senescence may be involved in this functional decline. The results of a correlative ultrastructural-morphometric assessment of number and regional density of synapses in the SON of aged and young rats will be presented.

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312.2 SP-IMMUNOREACTIVE VARICOSITIES LOCALIZED ON MEDULLARY TYROSINE HYDROXYLASE-IMMUNOREACTIVE CELLS BACKFILLED WITH HRP FROM THE SPINAL CORD. A.P. Nicholas*, M. B. Hancock*, and Sau-Wah Kwan*. (SPON: G. Russell). Department of Anatomy and Department of Human Biological Chemistry and Genetics, The University of Texas Medical Branch, Galveston, TX 77550.

Two-color immunoperoxidase staining was used to visualize black-stained substance P-immunoreactive (SPI) varicosities on amber-stained tyrosine hydroxylase-immunoreactive (THI) cells in the ventrolateral medulla of adult rats in which the thoracic spinal cord was injected with HRP. Animals deeply anesthetized with Nembutal were given multiple, bilateral injections, (4-7 µl total) of 50% HRP (Sigma; type VI) in phosphate buffered saline (0.1 M; pH 7.4) into the thoracic spinal cord through a micropipette inserted between adjacent spinal laminae. After 24-27 hours, the rats were again anesthetized and perfused transcardially with ice-cold 4% paraformaldehyde in 0.1M phosphate buffer (PB; pH 7.4). The brainstem was removed and postfixed overnight in 4% paraformaldehyde in PB. Vibratome sections (25-30 µm) were reacted with 0.035% diaminobenzidine (DAB), 2.5% nickel ammonium sulfate, and 0.01% $H_2O_2$ in 0.1M acetate buffer (pH 6.0) for 30 minutes. HRP-labeled cells showed fine, black granules in their cytoplasm. Sections containing HRP-labeled cells were washed and stored in PB for 24-48 hours. The tissue was then incubated overnight at room temperature in rabbit anti-SP (Immunonuclear; 1:5000), 0.2% Triton X100, and 1% normal goat serum in PB. The sections were then incubated in goat anti-rabbit IgG (1:100), rabbit PAP (1:640), and finally in the nickel-DAB solution used above. SPI processes were stained an intense black. The tissue sections were incubated overnight at room temperature in a 1:1000 dilution of a monoclonal antibody to TH. This was followed by incubations in rabbit anti-mouse IgG (1:100), mouse PAP (1:320), and finally in 0.05% DAB and 0.01% $H_2O_2$ in PB. Amber-stained THI cells enmeshed in SPI processes were observed in the ventrolateral medulla and in the solitary nucleus. Selected thick sections were embedded in Spurr plastic. Blocks of the ventrolateral medulla were cut from these sections and mounted on plastic rods. Semi-thin (1.4-1.8 µm) sections of this material were then cut using an ultramicrotome and glass knives. In these sections, black, SPI beads could be visualized along the borders of individual THI cells. Many of the THI cells were labeled with HRP, indicating that they projected to the spinal cord. The results suggest that SP released from fibers in the medulla may influence activity in brainstem catecholaminergic neurons that send fibers into the spinal cord.

312.3 INTERACTION OF SYMPATHETIC AND PARASYMPATHETIC NEURONS IN THE CARDIAC GANGLION OF NECTURUS. R.L. Parsons and D.S. Neel* Dept. of Anatomy and Neurobiology, University of Vermont, Burlington, VT 05405.

Components of the autonomic nervous system (ANS) regulate the function of many visceral structures. Many organs, such as the heart are dually innervated by both sympathetic and parasympathetic postganglionic fibers. Interaction between these two components of the ANS is known to occur in brain stem and spinal cord nuclei as well as at the target tissue. Recently, evidence has been presented suggesting an interaction between sympathetic and parasympathetic components within peripheral autonomic ganglia in the gut. A direct interaction between sympathetic and parasympathetic elements in a cardiac parasympathetic ganglion is presented in this study.

Experiments were done using acutely dissected or organ cultured parasympathetic cardiac ganglion preparations from Necturus masculosus (mudpuppy). Whole mount preparations of the septal tissue sheet which contained the parasympathetic ganglion were removed and pinned out on Sylgard coated petri dishes. The glyoxylic acid induced fluorescence technique was used to visualize catecholamine containing cells and fibers.

Numerous long brightly fluorescent varicose fibers form a complex network over clusters of parasympathetic ganglion cells and strands of cardiac muscle. In addition to these fibers, there are numerous small brightly fluorescent interneurons (SIF cells) interspersed between individual parasympathetic ganglion cells. Long fibers and processes from the interneurons join to form bundles which arborize over groups of parasympathetic cells. In peripherally located smaller groups of ganglion cells there are no interneurons, but these parasympathetic cells receive innervation from the long continuous fluorescent axons.

Two experimental procedures were applied to support the conclusion that these long fibers were indeed sympathetic postganglionic axons: a) explants of cardiac ganglia were maintained for varying times to produce degeneration of any severed axons; b) chemical sympathectomy was produced by injection of 6-hydroxy dopamine. The intrinsic SIF cells were apparently uneffected by both procedures. After eight days in culture or after 6-OH dopamine treatment all of the long continuous brightly fluorescent fibers, which normally intermingle with clusters of ganglion cells or innervate cardiac muscle, were absent. This indicates their extra-ganglionic origin. All of the isolated groups of parasympathetic ganglion cells not containing SIF cells were totally devoid of any catecholamine containing fibers.

312.4 SUBSET OF ASTROCYTES IN CEREBRAL CORTEX IN DISSOCIATED CELL CULTURE ACCUMULATE GLYCOGEN. P. A. Rosenberg* and M. A. Dichter(SPON: H. Blume). Department of Neurology, Children's Hospital, Boston, Mass. 02115.

Previous reports have demonstrated that norepinephrine (NE) decreases the accumulation of glycogen (GN) in the mouse cortical slice preparation. To identify the cells in which this effect of NE might be taking place, we used cerebral cortex in dissociated cell culture, and combined GN cytochemistry with anti-GFAP immunochemistry. Cultures of E15 rat cerebral cortex were prepared as previously described from this laboratory, and were used at 3-8 weeks. Cultures were fixed in ethanol and stained for glycogen using a modified periodic acid-Schiff technique. They were then incubated with a polyclonal rabbit anti-GFAP serum (courtesy Dr. A. Bignami), washed, and incubated with FITC-conjugated goat anti-rabbit antibody. Two types of nonneuronal cells in the cultures contained GN. One type of GN containing cell formed distinct plaques of cells which were GFAP negative and in which most cells contained GN. These plaques were also GFAP negative when they were not stained for GN, and were not always present in the cultures. The second GN containing cell type resembled astrocytes and were more specifically identified by anti-GFAP staining. Most of these cells appeared to be clearly GFAP positive (greater than 50 %). False negatives for GFAP staining are expected because fuchsin acts as an effective filter for the green emission of fluorescein, and therefore may prevent detection of the antibody in some cells. The GN containing astrocytes were typically found at the periphery of neuronal aggregates, occasionally within such aggregates, or near small groupings of solitary neurons. The overwhelming majority of astrocytes did not contain GN. Glycogen accumulating astrocytes therefore represent only a small subset of astrocytes in the cultures. These findings complement the recent observation that a subset of astrocytes in culture have beta receptors, determined by radioligand autoradiography (McCarthy, K. D., J. Pharm. Exp. Ther., 226: 282, 1983). Since we were able to demonstrate GN in the cultures, as well as its cellular localization, we attempted to demonstrate GN accumulation biochemically, as well as the effect of NE on this parameter. Cultures incubated with 3H-glucose demonstrated NE sensitive 3H-GN accumulation. The EC50 was 4 nM, with a beta receptor specificity. Together, these results suggest that at least one cellular target for NE in cerebral cortex is a specialized subset of astrocytes which accumulate GN subject to beta-adrenergic control.

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312.5 DOPAMINE ENHANCED TERMINAL EXCITABILITY OF HIPPOCAMPAL-ACCUMBENS NEURONES MEDIATED BY D-2 RECEPTORS. C.R. Yang* and G.J. Mogenson (SPON: D.L. Jones). Dept. of Physiology, Univ. of Western Ontario, London, Canada N6A 5C1.

The nucleus accumbens (NAC) receives converging glutamatergic afferents from the ventral subiculum of the hippocampus (HIPP) and dopaminergic afferents from the ventral tegmental area (VTA). Earlier we have reported that conditioning stimulation of VTA mesolimbic dopaminergic (DA) neurones attenuated the excitatory responses of NAC neurones to HIPP stimulation (Yang, C.R. & Mogenson, G.J., Brain Res., 324:69, 1984). The possibility that this attenuation is due to DA from the VTA neurones acting presynaptically on the terminals of the HIPP-NAC neurones was investigated with extracellular single unit recording technique. In urethane-anesthetized rats, antidromic action potentials recorded in the HIPP by glass microelectrodes were elicited by stimulating the medial NAC with the 4M NaCl-filled centre barrel of a four-barrelled micropipette assembly. The side barrels contained drug and DA solutions for iontophoresis onto the terminal regions of the identified HIPP-NAC neurones. The stimulating current was adjusted to elicit antidromic responses of HIPP neurones on 30-40% of trials which was considered to be the basal firing index.

Conditioning VTA stimulation (5-10 trains of 10 Hz pulses, 200-600 μA, 0.2 ms pulse duration), or iontophoretic application of DA (20-100 nA) produced a prolonged (mean, 25 min) enhancement of the firing index in 78/110 (71%) and 36/70 (51%) neurones, respectively. Twelve out of 33 (36%) of the neurones tested responded to both treatments. Iontophoretic application of LY-171555, a selective dopamine D-2 agonist, also produced a similar enhancement of firing index in 17/31 (55%) neurones tested. Iontophoretic application of sulpiride (a selective D-2 antagonist, n=7), but NOT SCH-23390 (a selective D-1 antagonist, n=4), blocked the increased firing index resulting from conditioning VTA stimulation. In order to rule out the possibility that the excitability changes produced by the DA input was mediated trans-synaptically, or by interneurones in the NAC, ibotenic acid lesions were made in the NAC of 15 rats 7-9 days before each recording session. Conditioning VTA stimulation and iontophoretic application of DA also produced an enhanced firing index in equal proportion of the HIPP-NAC neurones in these animals, compared to untreated animals.

The above evidence suggests that DA, via its D-2 receptors, exerts a depolarizing action on the terminals of HIPP-NAC neurones to increase their terminal excitability. This action of DA may constitute one of the presynaptic events which reduce excitatory transmitter release from HIPP-NAC neurones.
(Supported by MRC of Canada).

312.6 AN IN VITRO STUDY OF NOREPINEPHRINE EFFECTS ON LATERAL HYPOTHALAMIC NEURONAL RESPONSES TO GABA. J-T. Cheng, S.A. Azizi, J.K. Chapin and B.D. Waterhouse. Dept. Cell Bio., Univ. Tx Health Science Center, Dallas, TX 75235.

Previous studies from this laboratory have demonstrated that norepinephrine (NE) can consistently augment synaptically-mediated (70%) and GABA-induced (69%) inhibitory responses of lateral hypothalamic (LH) neurons in vivo. The present experiments further characterize the interactions of NE with LH neuronal responses to GABA and attempt to validate the LH slice preparation as a model for future intracellular studies of NE modulatory phenomena. Extracellular activity of LH cells was recorded from diencephalic slices (450 μm) incubated in ACSF at 33° C. Interactions of iontophoretically applied NE, isoproternol (ISO) or phenylephrine (PE) with responses of LH neurons (n=38) to microiontophoretic GABA were characterized using computer-generated ratemeter and histogram records. We observed dual actions of NE on spontaneous activity (SA) and GABA-induced responses of LH neurons. In 8 of 33 cells tested (24%), locally applied NE markedly enhanced inhibitory responses to GABA iontophoresis. GABA inhibition was augmented by NE at doses which had little (<10%) or no effect on SA in 5 of these 8 cases. In 19 cells (58%), iontophoretic application of NE antagonized GABA responses. In 9 of these cases, NE also had an excitatory effect on SA. Overall, in those cells where NE administration increased SA, it either antagonized or had no effect on GABA inhibition. In contrast, spontaneous firing rate was never elevated above control levels in those cases where NE potentiated GABA responses. Additional experiments demonstrated that iontophoretic application of the β agonist, ISO, routinely suppressed SA of LH neurons (n=4) and mimicked the facilitating action of NE on GABA in 3 cases. On the other hand, local administration of the alpha agonist, PE, produced NE-like antagonism of GABA inhibition and exerted an excitatory effect on SA in 3 of 3 cases tested. In addition, the antagonistic effect of NE on GABA responses was not blocked by application of the β antagonist, sotalol. In summary, these findings indicate that the commonly observed in vivo phenomena of NE augmentation of GABA and suppression of LH neuron spontaneous firing can be demonstrated in vitro and result from an interaction with β adrenoceptors. However, NE antagonism of GABA inhibition and excitatory effects on SA were more routinely observed in LH slices and appear to be mediated by alpha receptor activation. Thus, while the LH slice preparation appears to be a useful model for further study of NE/GABA interactions; a comparison of our present and past results emphasizes discrepancies between in vivo and in vitro data and brings into question the in vitro viability of the mechanism(s) responsible for NE enhancement of GABA responses. (Supported by AFOSR-85-0155, NINCDS NS 18081, the Klingenstein Foundation)

312.7 BIOGENIC MONOAMINES AFFECT A SLOW LIGHT-EVOKED ELECTRICAL RESPONSE OF THE RETINAL PIGMENT EPITHELIUM. S.M. Dawis* and G. Niemeyer*. (SPON: J. Fohlmeister). Neurophysiology Laboratory, Department of Ophthalmology, Universitaetsspital, CH-8091 Zuerich, SWITZERLAND.

Biogenic monoamines may be involved in controlling the function of the retinal pigment epithelium (RPE), a single layer of epithelial cells between the photoreceptors and the choroidal circulation. For example, monoamines have been implicated in the circadian rhythm of phagocytosis of photoreceptor outer segment discs by the RPE (Remé, C. et al., Brain Res., 298:99, 1984).

We found that biogenic monoamines can affect the maintained and light-evoked electrical activity of the mammalian RPE. The maintained activity of the RPE in the isolated, arterially perfused cat eye was indirectly monitored with an electrode in the vitreous referenced to an electrode at the posterior pole of the eye. The recorded standing potential (SP) is largely due to a difference in the potentials of the apical and basal membranes of the RPE. A 1 min pulse of diffuse white light (70 cd/$m^2$) was used to evoke a light peak (LP), a slow increase in the SP that reached a maximum amplitude of a few millivolts approximately 6 min after light onset. The LP has been shown to arise from a depolarization of the basal membrane of the RPE (Griff, E.R. and Steinberg, R.H., J. Physiol., 331:637, 1982; Linsenmeier, R.A. and Steinberg, R.H., J. Physiol., 331:653, 1982). At 20 micromolar, dopamine, norepinephrine, serotonin, and melatonin caused an increase in the SP of the eye and abolished the LP; histamine had no effect. These effects were reversible and were mimicked by dibutyryl cyclic AMP.

It appeared as if the monoamine-induced increase in SP saturated the system responsible for generating the LP. One possibility is that either the retinal dopaminergic or serotonergic system is involved in the generation of the LP. Another possibility is that the LP is generated by another retinal mechanism but that the RPE is influenced by monoamines released by the retina or present in the choroidal circulation. Perhaps the observed action of monoamines on the LP is the basis for the circadian rhythm seen in the clinical electrooculogram, an estimate of the amplitude of the human LP (Anderson, M.L. and Purple, R.L., Invest. Ophthal. Visual Sci., 19:278, 1980)

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312.8 ELECTROPHYSIOLOGICAL STUDIES OF MONOAMINE SENSITIVE NEURONS IN THE RAT PREFRONTAL CORTEX. S. R. Sesack and B. S. Bunney, Depts. Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06510.

Spontaneously active neurons in the medial prefrontal cortex (PFC) of the rat were investigated using single unit extracellular techniques. Animals were prepared for recording by a low cerveau isole transection performed under temporary halothane anesthesia. Microiontophoretic application of drugs through five barrel micropipettes was used to test the sensitivity of prefrontal neurons to monoamines and other neurotransmitters.

The majority of cells throughout the PFC were sensitive to the effects of norepinephrine (NE) and responded with an inhibition of firing rate. Dopamine (DA) also produced inhibitory responses throughout the PFC, however cells exhibiting the greatest sensitivity to dopamine were located primarily in the deepest layers. Serotonin (5HT) sensitive neurons could also be found in the medial PFC and while inhibitory responses to 5HT were common, excitatory responses were also occasionally observed. Several cells in the medial PFC were located which exhibited sensitivity to all three of the monoamines tested.

In addition to monoamines, neurons in the medial PFC were tested for their sensitivity to the neuropeptide cholecystokinin-octapeptide sulfate (CCK-8). Prefrontal neurons were equally divided between those showing an excitatory response to CCK-8 and those failing to respond at all. As previously reported, most DA sensitive cells within the deep cortical layers were sensitive to the excitatory effects of CCK-8 (Chiodo and Bunney, Science 219: 1449, 1983). A few CCK sensitive neurons, however, did not respond to any of the monoamines tested.

During the course of this investigation it became evident that cells in the medial PFC could be divided into 2 broad categories based on their extracellular waveforms. Both types of cells exhibited a biphasic action potential which displayed either an initial positive or an initial negative going segment. Both positive and negative going cells displayed similar sensitivities to the monoamines and CCK. However most of the cells in the deep layers which were primarily sensitive to DA exhibited negatively going waveforms, and the cells which were sensitive to both DA and CCK were also of the negatively going type.

These preliminary results suggest that cells within the medial PFC can be characterized both on the basis of sensitivity to various neuroactive substances and extracellularly recorded waveform. In addition, several cells in the PFC have been shown to respond to more than one transmitter. Experiments are in progress to investigate possible modulatory interactions among the monoamines, peptides, and classical neurotransmitters.

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312.9 NOREPINEPHRINE (NE) MEDIATES A SLOW HYPERPOLARIZING SYNAPTIC POTENTIAL (S-HSP) IN PARASYMPATHETIC NEURONS. P.Shinnick-Gallagher, T. Akasu*, J.P. Gallagher and K. Hirai*. Dept. of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX.

In cat parasympathetic ganglia of the urinary bladder, adrenergic fibers encircle and apparently terminate on cholinergic neurons. In the present study we analyzed the synaptic mechanism of the adrenergic innervation using intracellular recording techniques. Supramaximal stimulation of the preganglionic nerve trunk in the presence of hexamethonium (0.5mM) elicited a slow-inhibitory post-synaptic potential (s-i.p.s.p.). Increasing the stimulus intensity revealed the presence of a slower wave on the falling wave of the s-i.p.s.p. Treatment with atropine (0.1μM) blocked the s-i.p.s.p. and unmasked a s-h.s.p. This potential was reversibly abolished in low Ca solution suggesting release of a chemical transmitter. Yohimbine (0.1-1μM), an alpha$_2$-adrenoceptor antagonist, markedly depressed the s-h.s.p. in 56% of the neurons but even higher concentrations (3μM) did not affect s-h.s.p.s in 36% of the neurons. Recently we reported the existence of an adenosine-mediated s-h.s.p. in these neurons. S-h.s.p.s resistant to yohimbine were blocked by caffeine, a $P_1$-purinoceptor antagonist. Bath application of norepinephrine (50μM) produced a membrane hyperpolarization which was also markedly depressed by yohimbine (0.5μM). • Imipramine augmented the amplitude and duration of the caffeine resistant s-h.s.p. and the NE hyperpolarization. These data suggested the pharmacology of caffeine-resistant s-h.s.p. and NE hyperpolarization were similar. Membrane resistance was decreased 40% during the caffeine-resistant s-h.s.p. and 45% during the hyperpolarization produced by bath application of NE (50μM). This s-h.s.p. decreased in amplitude as the membrane was hyperpolarized and reversed polarity around -106mV. Similarly, the NE hyperpolarization decreased in amplitude with membrane hyperpolarization and reversed polarity around -108mV. In high K solution the reversal potentials of the s-h.s.p. recorded in caffeine and NE hyperpolarization shifted to more positive levels, around -78mV and -80mV, respectively. These data indicated that the caffeine-resistant s-h.s.p. and NE hyperpolarization are mediated by an increase in K conductance. These data demonstrate that a non-cholinergic, non-purinergic hyperpolarizing response elicited by preganglionic nerve stimulation in neurons of vesical parasympathetic ganglia is most closely mimicked by the response to exogenously applied NE. In addition, the catecholamine mediated s-h.s.p. produced an inhibition of spontaneous activity in vesical neurons which is ten times longer than the inhibition produced during the s-i.p.s.p. These data suggest that the catecholamine-mediated s-h.s.p. may be the underlying synaptic substrate for the inhibition of transmission in vesical ganglia observed during sympathetic nerve stimulation. (Supported by NS16228)

312.10 **DIFFERENTIAL ORGANIZATION OF THE EFFERENT PROJECTIONS OF THE VENTROMEDIAL MESENCEPHALIC TEGMENTUM IN THE CAT.** T. Scheibner* and I. Törk* (SPON: D. J. Tracey) School of Anatomy, University of N.S.W., Kensington 2033, Sydney, Australia.

The cortical and subcortical projections of the ventromedial mesencephalic tegmentum (VMT) in the cat were studied using the retrograde axonal transport of the highly sensitive tracer, WGA-HRP. One ul of a 1.5% solution of the tracer was injected into the motor, somatosensory, auditory, visual, prefrontal, insular, entorhinal, cingulate and parasplenial cortical areas and into the accumbens nucleus and surrounding septal structures.

An extensive projection was observed to all cortical regions studied with the largest numbers of retrogradely labelled cells being found in the VMT following prefrontal, cingulate, visual and insular cortical injections. Further, the VMT nuclei were observed to contribute differentially to these projections. The largest numbers of retrogradely labelled cells were found in the linearis rostralis nucleus (LR) (25% of the total estimated ipsilateral population of LR, or 1200 neurons). The parabrachialis pigmentosus (PBP), the linearis centralis (LC) and the interfascicular (IF) nuclei contained progressively fewer retrogradely labelled cells. The paranigralis nucleus (PN) was not found to project to any cortical region studied. However, following injection of the accumbens nucleus, the PN contained the largest numbers of retrogradely labelled cells (50% of the total estimated ipsilateral population of PN, or 4000 neurons). The PBP, IF and LC contained progressively fewer labelled cells, and in the LR only 5% of the total estimated ipsilateral population of this nucleus was labelled. Septal and accumbens nucleus injections resulted in the same distribution of labelled cells (concentrating in the ventrocaudal part of the VMT) even though accumbens nucleus injections yielded the largest numbers of labelled cells.

These results indicate that in the cat the cortical projections of the VMT are much more extensive than described in the rat. Furthermore, we observed a strong topographical organization of projections. The VMT neurons which project to subcortical limbic structures are found ventrocaudally, whereas neurons projecting to cerebral cortex are found dorsorostrally. Finally, we identified one population of neurons, found in PN, which does not project to cerebral cortex.

312.11 COMPARATIVE ANATOMY OF THE MESENCEPHALIC VENTROMEDIAL TEGMENTUM. G. Halliday* and I. Törk* (SPON: R. Bandler). School of Anatomy, University of N.S.W., Kensington, 2033, Sydney, Australia.

Nissl preparations of the mesencephalic ventromedial tegmentum (VMT) in four species (rat, cat, monkey and human) were qualitatively and quantitatively compared. In all species five subnuclei could be distinguished: nucleus parabrachialis pigmentosus (PBP), nucleus paranigralis (PN), nucleus interfascicularis (IF), nucleus linearis rostralis (LR) and nucleus linearis centralis (LC). The relative position and extent of these nuclei are illustrated in three dimensional reconstructions from serial sections. The size and number of cells in each nucleus vary between the different species. The data obtained from these nuclei are compared to data from the substantia nigra pars compacta (SNC).

**Extent and Cell Number.** The majority of VMT cells are located laterally in PBP and PN. These lateral nuclei also make up the bulk of the VMT volume. The greatest variation between species in nuclear shape and extent is seen in the medially located LC and LR. LR, although seen in all species, was substantial only in cat. The VMT is small in the monkey, relative to both other species and its own SNC.

| Species | rat | cat | monkey | human |
|---|---|---|---|---|
| VMT volume($mm^3$) | 1.20 | 14.38 | 6.50 | 183.40 |
| SNC volume($mm^3$) | 0.34 | 3.20 | 6.28 | 67.60 |
| VMT cell no. | 27215 | 62691 | 47497 | 690420 |
| SNC cell no. | 11878 | 26027 | 71086 | 436409 |

**Cell Density.** In all cases, the SNC is more densely packed with cells than the VMT. The rat has the highest cell packing density in the SNC and VMT. Among the nuclei of the VMT, IF is the most densely packed. With the exception of the cat, cell densities are higher laterally than medially in the VMT. The table below gives the cell packing density in the VMT and SNC, as cell density x mean soma diameter.

| Species | rat | cat | monkey | human |
|---|---|---|---|---|
| VMT density(cells/$mm^2$) | 309 | 74 | 80 | 120 |
| SNC density(cells/$mm^2$) | 561 | 187 | 260 | 200 |

**Cell Size.** The cells of the VMT are heterogeneous in size and staining properties. In general, the VMT cells are smaller than those found in the SNC. The human has the largest VMT cells and the rat has the smallest VMT cells. IF is made up of the most homogeneous group of small cells in all species and LR has the greatest proportion of large cells in all species.

| Species | rat | cat | monkey | human |
|---|---|---|---|---|
| VMT cell size(um) | 13±3 | 15±7 | 16±5 | 26±9 |
| SNC cell size(um) | 16±4 | 23±5 | 23±5 | 31±7 |

Our findings indicate that the disposition and shape of the VMT and its subnuclei vary across species, and these differences are further reflected in the quantitative analysis.

312.12 EFFECT OF ACUTE AND CHRONIC HALOPERIDOL ON MID-BRAIN DA CELLS IN UNANESTHETIZED RATS. G. Mereu, S.P. Han* and R.Y. Wang (SPON: E. Rubin). Department of Pharmacology, St. Louis University School of Medicine, St. Louis, MO 63104.

Studies from this and other laboratories have shown that acute administration of antipsychotic drugs (APD's) such as haloperidol (HAL) increases whereas prolonged administration (3-4 weeks) reduces in a time-dependent manner the number of spontaneously active dopamine (SA-DA) cells of both $A_9$ and $A_{10}$ areas. Several lines of evidence suggest that such effect reflects a progressive depolarization inactivation induced by the drugs on mid-brain DA neurons. Because the time course of such effect, particularly for $A_{10}$ cells, is well correlated with the clinical response of APD's, it has been proposed that the decrease SA-DA cells might explain the delayed therapeutic effects of the prolonged treatment with neuroleptics. The above studies were performed on chloral hydrate (CH) anesthetized rats. However, differences in the basal activity and in the responses to drugs of DA neurons have recently been reported between anesthetized and unanesthetized rat preparation. Therefore the present experiments were designed to investigate whether anesthesia was affecting the changes in SA-DA cells to acute and chronic HAL treatment.

Under CH anesthesia rats had chronically implanted a device by which the head could be retained in the stereotaxic apparatus without pain. The animals were then adapted to the restraint in a hammock jacket tightly wrapped around the body to minimize the struggling, 2-3 hours each day for 2 weeks. Recording electrodes were lowered in the $A_9$ or $A_{10}$ area through a plastic cylinder which sat on top of the hole previously drilled in the skull.

The preliminary results indicate that in both $A_9$ and $A_{10}$ areas, the number of SA-DA cells is significantly higher in this preparation than CH anesthetized rats (1.45±0.16 vs 0.87±0.03, $P<0.001$ for $A_9$ and 2.34±0.21 vs 1.53±0.04, $P<0.001$ for $A_{10}$). However, acute and prolonged administration of HAL (0.5 mg/kg/day) increases and decreases, respectively, the SA-DA cells (see table) in a fashion similar to that previously observed in CH preparation. In two rats apomorphine (100 µg/kg s.c.) administered after 4 weeks of HAL administration, reversed the SA-DA cells to values close to baseline suggesting that the decrease in SA-DA cells was due to an excessive depolarization by APD's.

In conclusion, the study supports and extends our previous results to unanesthetized preparation.

Time-course of DA cells/track after HAL

| | | | Weeks of treatment | | | |
|---|---|---|---|---|---|---|
| Area | Baseline | Acute | 1 | 2 | 3 | 4 |
| $A_9$ | 1.45±0.16 | 2.01±0.18 | 1.49±0.26 | 1.08±0.16 | 1.33±0.21 | 0.53±0.08 |
| $A_{10}$ | 2.34±0.23 | 4.30±0.48 | 2.50±0.19 | 2.13±0.12 | 1.90±0.13 | 1.12±0.20 |

(Research supported by USPHS grants MH34424, MH38794 and MH00378.)

312.13 COMPARISON OF EFFECTS PRODUCED BY CHRONIC ADMINISTRATION OF d-AMPHETAMINE, HALOPERIDOL AND CLOZAPINE ON THE ACTIVITY OF LOCUS COERULEUS NEURONS. O.A. Ramirez* and R.Y. Wang (SPON: M. Meldrum). Department of Pharmacology, St. Louis University School of Medicine, St. Louis, MO 63104.

The role of norepinephrine (NE) in the pathophysiology of schizophrenic symptoms has been controversial. To determine the participation of noradrenergic transmission during the schizophrenic symptomatology, effects produced by prolonged treatment with d-amphetamine (d-AMP) or antipsychotic drugs (APD's) such as haloperidol (HAL) and clozapine (CLOZ) on NE-containing neurons in the locus coeruleus (LC) were studied.

Groups of male rats received intraperiotoneal injections of d-AMP (5 mg/kg) twice daily for 7 days. Control rats received daily injections of saline. Single unit recordings of NE cells in the LC were obtained 24 hrs after the last injection. After chronic d-AMP (CAMP) treatment the number of spontaneously active cells/track was decreased significantly. Interspike interval histograms of NE cells showed a "disorganized" firing pattern after CAMP treatment. On the other hand, $\alpha_2$ adrenergic receptors became subsensitive to i.v. d-AMP or iontophoretic (IONTO) application of DA (0.1 M) or clonidine (CLON) (0.01 M). CAMP treatment significantly increased the dose of d-AMP required to suppress the firing of LC NE neurons. ID50s for d-AMP in CAMP and control rats were 280.8±40.7 and 55.2±12.2 µg/kg ($P<.001$), respectively. CAMP treatment also increased markedly the ejection current (nA) of CLON and DA necessary for inhibiting the activity of LC NE neurons. The inhibitory effects of DA in LC cells is not mediated by DA receptors, since trifluperazine, an antagonist of DA receptors, was not able to reverse the inhibition produced by DA.

Chronic HAL treatment (0.5 mg/kg subcu. for 3-5 wks) did not change the number of spontaneously active cells/track but markedly decreased the mean firing rate of these cells. On the other hand, chronic CLOZ (20 mg/kg subcu.) produced an increase in both the firing rate and the number of spontaneously active LC cells/track.

In conclusion, the results suggest that during CAMP treatment, which is often associated with schizophrenic symptomatology, the NE transmission is decreased. In addition, because neither HAL nor CLOZ, after prolonged administration, induced a time-dependent reduction of spontaneously active NE cells in the LC, our results do not support the view that a significantly increased NE activity in the brain caused the schizophrenic symptoms. On the other hand, the marked increase of NE activity after chronic CLOZ treatment could contribute to the selective action of CLOZ on $A_{10}$ mesolimbic (particularly at the terminal regions) but not $A_9$ nigrostriatal system. (This research was supported by USPHS grants MH-34424, MH38794 and a RSDA MH-00378. O.A.R. was supported by a Fellowship from the CONICET of Argentina.)

312.14 A METHOD FOR COMBINED LOCAL DRUG INFUSION AND ELECTROPHYSIOLOGICAL RECORDING OF LOCUS COERULEUS NEURONS. L.M. Adams and S.L. Foote, Department of Psychiatry, UCSD, La Jolla, CA 92093

While it is of interest to determine the effects on target cells of changes in the discharge rate of locus coeruleus (LC) neurons, it is difficult to selectively activate these neurons using electrical stimulation; the LC is limited in size and is surrounded by major fiber tracts originating from other nuclei. The high activation threshold of LC axons also makes it difficult to selectively activate them at other locations. Finally, while it would be useful to examine the effects of acute, reversible LC "lesions", it is difficult to inhibit these neurons by electrical means.

We have developed a method for manipulating the electrophysiological activity of LC neurons in a reversible, bi-directional, verifiable way using a combined cannula and microelectrode assembly to record LC unit activity while infusing drugs. A stainless steel microelectrode is cemented parallel to a piece of 27 ga hypodermic tubing (guide canula) which terminates 3 mm above the microelectrode tip. A 33 ga needle with a 27 ga sleeve is connected to a 10 µl syringe by PE tubing. When inserted, the needle protrudes to the same depth as the microelectrode , with 400 µm lateral displacement. Rats were placed in a stereotaxic apparatus under halothane anesthesia. A glass micropipette was used to localize LC neurons, then removed, and the electrode/infusion assembly lowered at the same coordinates The infusion line was pre-filled with a sequence of different drugs and or doses (separated by air bubbles) and the infusion assembly lowered with the needle in place. After baseline firing and sensory responses were established, the "drug series" was infused at a rate of 0.05 µl per min. Finally, electrical current was passed through the electrode to mark the recording site; and Pontamine sky blue dye (0.1 µl) was infused to mark the injection site. The "Prussian blue reaction" was used to produce an intense green spot at the recording site. When these sites were within of the locus coeruleus, dye injections were usually within the mesencephalic nucleus of V.

Infusion of 0.1 µl of 0.02M acetylcholine (ACH) produced a 3-4 min long increase in LC firing rate with a 1 min latency to onset. Larger volumes (0.15 µl) produced greater increases in firing rate lasting 10-12 min. ACH effects were readily reversed with equimolar doses of scopalamine (SCOP). Conversely, 0.6 µl of 0.02M SCOP antagonized the effect 0.1 µl "pulses" of ACH for up to 4 min. The effects of 0.02 M ACH were also rapidly reversed by equal volumes of 0.001 M clonidine. Scopalamine and clonidine reduced basal firing rates without blocking responses to sensory stimuli.

This method for producing verified, quantified activation or suppression of LC neuronal activity will be useful in studies correlating LC activity with behavior or target cell activity. Supported by NS21384, MH4008, Dystonia Med Res Fdn.

312.15 THE FACILITATING ACTION OF LOCUS COERULEUS ACTIVATION ON CA1 PYRAMIDAL CELL EXCITABILITY AND ON TRANSCALLOSALLY ELICITED CORTICAL FIELD POTENTIALS IN VIVO. H.-R. Olpe*, R.S.G. Jones*, J. Laszlo*, P.C. Waldmeier* and L. Maître* (SPON: G.Gmelin), CIBA-GEIGY Ltd., Pharmaceuticals Division, CH 4002 Basel, Switzerland

A depressant action of iontophoretically applied noradrenaline on hippocampal and cortical spontaneously active neurons has been described in vivo. In vitro however, low concentrations of noradrenaline have a disinhibitory effect on hippocampal CA1 neurons (Müller et al. 1981; Madison and Nicoll, 1982; Haas and Konnerth, 1983). In the present study locus coeruleus (L.C.) neurons were activated by injecting small volumes (0.2-2.0 µl) of physiological saline solution containing 0.5 mM glutamate into the area of L.C. by means of a hamilton syringe. Local Glu-injections afford a selective activation of L.C. neurons without affecting passing fibres. Glu-activation of L.C. induced a reversible increase in the CA1 population spike amplitude without altering the slope of the negativity of the epsp recorded in the stratum radiatum. In DSP-4 treated, noradrenaline-depleted animals, the facilitatory effect was abolished.

A multiphasic cortical field potential (TPC) was recorded in the sensorimotor cortex of the anaesthetized rat induced by electrical stimulation of the contralateral homotopic area. The amplitude of the first negativity of the TCP was reversibly increased by local Glu-injections into L.C. This facilitatory effect was strongly reduced in DSP-4 treated, noradrenaline depleted animals. The amplitude of the first negativity was also increased following injections of hydergine (10 mg/kg i.p. / 4 out of 6 experiments). Hydergine is a potent activator of L.C. neuronal activity (Olpe and Steinmann, 1982).

In conclusion, the present findings demonstrate that L.C. activation facilitates the responsiveness of cortical neurons to excitatory, probably glutamatergic inputs.

Müller, A.L. et al., Brain Res. 214, 113-126 (1981). Madison, D.V. and Nicoll, R.A., Nature 229, 636-638 (1982). Haas, L.H. and Konnerth, A., Nature 302, 432-434 (1983). Olpe, H.-R. and Steinmann, M.W., J. Neural Transmission 55, 101-109 (1982).

312.16 CARDIOVASCULAR AND CENTRAL NORADRENERGIC EFFECTS OF INTRAVENOUS COCAINE ADMINISTRATION IN RATS. D.K. Pitts*, and J. Marwah (SPON: L.M. Yunger). Depts. of Pharmacology, Physiology and Neurobiology, Indiana Univ. Sch. Med., Terre Haute, IN 47809.

The present studies examined the cardiovascular effects of intravenous cocaine HCl in pentobarbital (65 mg/kg, I.P.) anesthetized rats. Mean arterial pressure (MAP) was recorded from the femoral artery, and heart rate was derived from the pulsatile blood pressure trace. Cocaine (0.16-5 mg/kg) administered intravenously (I.V., lateral tail vein) over a 30-second period produced an initial dose-dependent increase in MAP within seven to 25 seconds followed by a more prolonged (several minutes) depressor effect. This effect on MAP was accompanied by a significant increase in respiratory rate and bradycardia. Cocaine (1.25 mg/kg, I.V.) administered to conscious-restrained rats produced a pressor response similar to that seen in barbiturate anesthetized animals. The subsequent depressor phase was, however, greatly attenuated. Procaine (0.31, 1.25 mg/kg, I.V.) produced a depression of MAP similar to that seen with cocaine in the barbiturate anesthetized rat. This depressor effect of procaine (1.25 mg/kg, I.V.) was also greatly attenuated in the conscious-restrained animal. These results suggest that the local anesthetic properties of cocaine may be specifically responsible for the hypotensive effects of cocaine in barbiturate anesthetized animals. Preliminary results further suggest that the initial pressor response, but not the subsequent depressor response to IV cocaine is present in urethane (1.25 g/kg, I.P.) anesthetized rats. I.V. cocaine (1 mg/kg) decreased the spontaneous firing rate of single identified noradrenergic locus coeruleus neurons by approximately 50% in urethane anesthetized rats. This effect was reversed by the specific alpha-2-adrenoceptor antagonist, piperoxane. The duration of the effect on locus coeruleus neuron firing rate was significantly greater than that of the relatively brief pressor effect. Our results suggest that cocaine produces both specific (e.g., at locus coeruleus) and non-specific (local anesthetic) effects after systemic administration. In addition, these effects of cocaine are dependent upon the class of anesthetic employed. (Supported in part by a Grant-in-Aid, #83-757; an Established Investigatorship, #85-118, from the American Heart Association; and a grant from NIDA, #1-RO1-DA-03519.)

312.17 SYNCHRONOUS BURSTING OF LOCUS COERULEUS NEURONS IN TISSUE CULTURE: A COMMON EXCITATORY INPUT OR ELECTROTONIC COUPLING OF NEURONS? P.G. Finlayson* and K.C. Marshall. (SPON: J. Kucharczyk) Dept. of Physiology, Univ. of Ottawa, Ottawa, Canada, K1H 8M5.

Neurons in the Locus Coeruleus (LC), the largest noradrenergic nucleus in the CNS, appear capable of functioning in synchrony. In multiple unit recordings of LC neurons in rats recovering from anaesthesia (Akaike, T. Brain Res. 239: 629-633, 1982) or in unanaesthetized rats (Aston-Jones, G. & Bloom, F., J. Neurosci. 1: 887-900, 1981), synchronous bursts of activity are consistently observed in the two or more recorded neurons. In addition, negative field potentials (FP) temporally synchronized with bursts in LC neurons were also reported in these studies. As similar FPs are observed during paradoxical sleep, a state where LC neurons are quiescent and do not burst, Aston-Jones and Bloom suggested that the negative FP may reflect a concerted excitatory input onto LC neurons. In tissue slices, bursting of LC neurons has not been reported. However, LC neurons in explant cultures from mouse brain do exhibit synchronized bursting, and we have examined the mechanism of this synchronous activity.

Preparation of LC explant tissue cultures has been described (Hendelman et al., Dev. Neurosci. 5: 64-76, 1982). During simultaneous intracellular recordings of pairs of LC neurons in such cultures, bursting activity was monitored, and electrotonic coupling was tested by passing polarizing current pulses into either cell. In other studies, after intracellular injection of the fluorescent dye, Lucifer Yellow, into single LC neurons, cultures were examined for secondarily stained LC neurons (i.e. dye-coupling).

In simultaneous recordings of pairs of LC neurons from cultures 23 to 30 days in vitro, bursting activity in one neuron was always synchronized with bursting activity or barrages of depolarizing events in the second cell. These depolarizing events were synchronized in recorded cells and apparently summate to evoke a burst. In a few neurons asynchronous tonic activity was observed. With injection of current into either neuron in a pair, no current or voltage spread into the second cell was apparent, even when action potentials were elicited. A further indication that these cultured LC neurons are not electrotonically coupled is the lack of dye-coupling with intracellular injection of Lucifer Yellow. Thus, bursting activity in LC neurons appears to be due to a common excitatory input, and not to involve electronic coupling. This common excitatory input onto LC neurons in tissue culture must be derived from cells near or within the LC, as our explant cultures contain little brainstem tissue surrounding the LC.
Supported by the Medical Research Council of Canada.
PGF is supported by an Ontario Graduate Scholarship.

312.18 MORPHOLOGICAL CHARACTERISTICS OF CULTURED LOCUS COERULEUS NEURONS DEMONSTRATED BY INTRACELLULAR INJECTIONS OF HRP. K.C. Marshall, D.M.A. Richer* & P.G. Finlayson*, Dept. of Physiology, Univ. of Ottawa, Ottawa, Canada K1H 8M5.

Explant cultures of the locus coeruleus (LC) were prepared from brains of neonatal mice (Hendelman, W.J. et al., Dev. Neurosci. 5: 64-76, 1982). After culturing for 21-30 days, cells in tightly clustered groups having large pale nuclei and prominent nucleoli were injected by iontophoretic pulsing through intracellular micropipettes containing 4% horseradish peroxidase (HRP). Only cells having a stable membrane potential of at least -50 mV were injected. Membrane potential and spike generation by depolarizing pulses were monitored throughout the injection period. Most cells exhibited periodic bursting activity which was independent of the current injections. Cultures were incubated in physiological solution for 2 - 3 hours following the injection, fixed overnight and stained using the diaminobenzidine reaction.

Successfully stained cells had rounded cell bodies with 3-5 primary dendrites (about 1/3) or elongated somata with 2 or 3 dendrites (about 2/3). The rounded cells had usual diameters of 20-26 , while the elongated ones had minor and major diameters of 13-20 and 30-40 , respectively. The dendrites were usually 150-300 long and had relatively long (usually 10-20 ) spines. Spines were also observed on the somata of about half of the stained cells, but were short and stubby in comparison with those on the dendrites. The elongated cells with few dendrites seldom assumed a bipolar configuration, but rather had a V or U shape.

In about ½ of the well-stained cells, 2 or 3 axon-like processes were observed to emanate from the soma or primary dendrites. These axon systems were multiple-branching and fine in diameter. They generally gave rise to long-coursing projections within the explant and into the outgrowth zone, as well as local networks within the LC cluster or its immediate surroundings. Less than half of these cells had axonal networks which were characterized by prominent varicosities. The multiple axon-like processes observed in these cells are also observed in Lucifer-Yellow stained cells in these cultures. No systematic differences in morphological characteristics were noted in the younger vs. the older cultures.

The characteristics of these cells appear similar to those noted in several studies of LC neurons in the rat, with the exception of the multiple axon-like processes and the V or U shaped configuration of elongated cells. [There is however an observation of multiple axon-like processes on an LC neuron by Swanson (Brain Res. 110: 39-56, 1976)]. It is not clear whether these features are characteristic of the culture system or represent properties not easily observable in the histological preparations from animals.
Supported by the Medical Research Council of Canada.
PGF is suppported by an Ontario Graduate Scholarship.

313.1 A RAPID, QUANTITATIVE COLORIMETRIC BIOASSAY FOR NEURONOTROPHIC AGENTS. R. Fagnani,* M. Manthorpe, S.D. Skaper and S. Varon. Dept. Biology, Sch. of Med., Univ. Calif. San Diego, La Jolla, CA 92093.

The biological activity of neuronotrophic agents, such as Nerve Growth Factor (NGF) or Ciliary Neuronotrophic Factor (CNTF) is typically determined by (i) presenting serial dilutions of the putative trophic sample to replicate neuronal microcultures, (ii) fixation of the neurons after a selected time in vitro, (iii) microscopic counts of individual neurons, and (iv) construction of dose response curves. The titer of the test sample [in Trophic Units (TU)/ml] is defined as that sample dilution eliciting half-maximal neuronal survival. These neuronal bioassays are time-consuming and tedious, requiring about 90 min to count the neurons in a 96-well microtiter plate and calculate titers.

Here we describe a rapid colorimetric method, based on a previously described lymphocytic cytotoxic assay (J. Immunol. Methods 65: 55-63, 1983), to determine the number of viable neurons in microwells. This procedure involves setting up the serial dilutions and neuronal microcultures in the usual manner but introducing into the culture medium a vital dye, MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide]. This tetrazolium derivative is converted by viable neurons into a blue formazan product which can then be quantitated using an ELISA microplate reader. The amount of blue product which forms is directly proportional to the number of surviving neurons and can be used for the accurate determination of neuronotrophic titers. The microplate reader has been interfaced with a computer and various data output formats are available. The entire procedure of counting and titer calculation now requires only 2 min per microplate, a 50-fold reduction in time over the previous methods. Selected parameters and uses of this neuronotrophic bioassay will be presented.

Supported by NINCDS grant NS 16349.

313.2 INTERACTION OF BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) AND OTHER NEUROTROPHIC FACTORS IN THE REGULATION OF SENSORY NEURON SURVIVAL IN CULTURE. A.M. Davies, Y.-A. Barde and H. Thoenen, Dept. of Anatomy, St. Georges' Hospital Medical School, London SW17 0RE, U.K. and Dept. of Neurochemistry, Max-Planck-Institute for Psychiatry, D-8033 Planegg-Martinsried, FRG.

BDNF, a small basic protein purified from pig brain (Barde et al, EMBO J. 1:549-53 '82), has been shown to promote the survival of neurons isolated from the nodose and dorsal root ganglia, but is inactive on sympathetic and parasympathetic neurons (R.M. Lindsay, H. Thoenen and Y.-A. Barde, submitted). Here we report that BDNF is active on all populations of primary sensory neurons of the embryonic chick; these comprise the geniculate, vestibular, petrosal, jugular, nodose and dorsal root ganglia and the dorso-medial, ventro-lateral and mesencephalic neurons of the trigeminal nerve. BDNF promoted neuronal survival in dissociated cultures and elicited neurite outgrowth from explants. This effect was first evident between embryonic days 4 and 8 (E4 to E8) and was maximal between E10 and E12. To determine whether or not BDNF-responsive neurons also respond to other neurotrophic factors we have studied the trophic requirements of the exclusively proprioceptive trigeminal mesencephalic neurons. In glial-free culture, the survival of more than 70% of these neurons is promoted by a soluble extract prepared from their peripheral target tissue, skeletal muscle (A.M. Davies, submitted). A similar percentage survival was promoted by saturating concentrations of BDNF, and there was no additional survival in the presence of both BDNF and skeletal muscle extract. At subsaturating concentrations the effects of both agents in combination were additive. Both BDNF and skeletal muscle extract were maximally effective during the period of natural neuronal death. We propose that in vivo the survival of sensory neurons is dependent upon the combination of subthreshold amounts of two survival factors. In the case of trigeminal mesencephalic neurons, the central processes are exposed to BDNF and the peripheral ones to the skeletal muscle factor. Although this factor is not yet purified, we know that it is functionally distinct from nerve growth factor (NGF) and BDNF: whereas the skeletal muscle factor supports the survival of mesencephalic neurons, NGF does not in spite of the presence of NGF receptors (A.M. Davies and H. Rohrer, unpublished), and whereas BDNF promotes neurite outgrowth from the ventrolateral part of the trigeminal ganglion, the skeletal muscle factor does not.

313.3 PARTIAL CHARACTERIZATION OF A NOVEL NEUROTROPHIC FACTOR FROM PERIPHERAL NERVE TUMORS. S. Dostaler*, R.J. Riopelle, and P.M. Richardson. Department of Medicine (Neurology), Queen's University, Kingston, Canada K7L 3N6 and Division of Neurosurgery, McGill University, Montreal, Canada H3G 1A4.

Explants of peripheral nerve tumors (neurofibromas) from patients with Von Recklinghausen disease have been shown to release at least two neurotrophic factors one, of which is immunologically cross-reactive with mouse nerve growth factor (NGF) (Riopelle, R.J. and Riccardi, V.M., Neurology, 34, Supp. 291, 1984). Similar trophic factors are found in normal rodent peripheral nerve (Riopelle, R.J. et al., Neurosci. Lett., 25:311, 1981; Richardson, P.M. and Ebendal, T., Brain Res., 246:57, 1982).

A neurite-promoting factor with an apparent MW of 20,000 has been detected in extracts of a neurofibrosarcoma from a patient with Von Recklinghausen disease. On poly-D-lysine and laminin substrata the species, designated NFNTF, promotes neurite outgrowth from dissociated chick embryo sensory neurons that is not inhibited by antibody to NGF, and also from dissociated ciliary neurons. NFNTF was purified by ion exchange and gel filtration chromatography, followed by reverse phase high performance liquid chromatography (RPLC) on μBondapak C-18 in a mobile phase of acetonitrile in trifluoroacetic acid. On RPLC at least one biologically active fraction with retention time differing from that of NFNTF was found. The neurite-promoting activity of this latter fraction was completely inhibited by antibody to NGF.

Sheath cell tumors may prove to be a rich source of neurotrophic factors that are present in minute quantities in normal peripheral nerve tissue. (Supported by the PSI Foundation of Ontario, the Clare Nelson Bequest of the Kingston General Hospital, and MRC Canada).

313.4 ISOLATION OF MAMMALIAN PERIPHERAL NERVE CILIARY NEURONOTROPHIC FACTOR. M. Manthorpe, S.D. Skaper, L.R. Williams, H.J.L. Fryer, and S. Varon. Dept. of Biology, Sch. of Med., Univ. Calif. San Diego, La Jolla, CA 92093.

Past work, using E8 chick ciliary ganglionic (CG) neurons as test cultures, has lent support to the general hypothesis that innervation territories produce trophic factors which control the survival of their innervating neurons during and possibly after the developmental neuronal death period (for review, see Growth and Maturation Factors, Volume 3, G. Guroff, ed., 1985, pp. 77-117). One such factor, Ciliary Neuronotrophic Factor or CNTF, was recently purified from extracts of selected intraocular tissues containing the innervation territories of CG neurons (J. Neurochem. 43: 1468-1478, 1984). The purified chick eye CNTF has an $M_r$ = 20.4 kD and a pI = 5.0 and supports the survival of, in addition to cultured CG neurons, those from dorsal root and sympathetic ganglia. More recently we have recognized the adult rat peripheral nerve to be another very rich source of CNTF activity.

We report here the purification of this mammalian CNTF from extracts of adult rat sciatic nerve using a fractionation protocol nearly identical to that employed for the isolation of the avian eye CNTF. The purified nerve CNTF differs from the eye-derived one in molecular weight (24 kD) and isoelectric point (5.6) but is similar to eye CNTF in its stability to SDS-mercaptoethanol, ability to support embryonic E10 sensory and sympathetic, but not E8 sensory neurons, and non-immunoreactivity to antibodies raised against purified mouse submaxillary NGF.

Supported by NINCDS grant NS 16349.

313.5 CHARACTERIZATION AND PARTIAL PURIFICATION FROM PIG LUNG OF SURVIVAL-PROMOTING AND CHOLINE ACETYLTRANSFERASE (CHAT)-SUSTAINING ACTIVITIES FOR PARASYMPATHETIC NEURONS. T.L. Wallace and E.M. Johnson, Jr. Dept. of Pharmacology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

Pig lung, among many tissues examined, was found to be a potent source of Survival-promoting and ChAT-Sustaining activities for chick parasympathetic neurons in culture. The bioassay used to detect these activities involves incubating either intact embryonic (E) chick ciliary ganglia or dissociated neurons for two to three days with tissue culture media, 10% serum, 35 mM KCl and lung extract. Survival was judged by phase contrast microscopy and ChAT activity was determined by a radioenzymatic assay. The survival-promoting activity in a crude extract of pig lung (25,000 x g supernatant fraction) exerted its effects on chick parasympathetic neurons, whereas it had no effect on rat sympathetic, chicken and rat dorsal root sensory, or rat nodosal neurons. This survival-promoting effect on parasympathetic neurons was not due to an indirect action on non-neuronal cells since elimination of these cells using cytosine arabinoside C did not affect survival. The Survival-promoting and ChAT-Sustaining activities bore similar biological properties. Both: sustained ChAT or promoted survival of E7 to E12 chick ciliary neurons, but not neurons that were E14 to E18; were precipitated with the same concentrations of ammonium sulfate or organic solvents; were stable at -40°C, room temperature, 37°C, and to multiple freezing-thawing; were trypsin resistant; and eluted from chromatographic columns in the same fractions. High and low molecular weight (MW) species of each type of activity were found. The high MW species eluted with neuritic-promoting activity for dissociated neurons, whereas there was little or no neuritic-promoting activity eluted with the low MW species. In addition, the low MW species was trypsin resistant, and was eluted with very little $OD_{280}$, suggesting that a high degree of purification has been achieved if the material is a peptide. These findings suggest that one or several molecules exist in pig lung that express both Survival-promoting and ChAT-Sustaining activities, and that because of their specificity for parasympathetic neurons, may be different from previously reported trophic-like factors. This work was supported by a March of Dimes research grant and by a postdoctoral fellowship from the Muscular Dystrophy Association to T.L.W.

313.6 NEURONOTROPHIC ACTIVITIES IN THE TRANSMITTER-STORING ORGANELLE OF CHROMAFFIN CELLS. K. Unsicker and R. LIETZKE*. Dept. of Anatomy and Cell Biology, Univ. of Marburg, Robert-Koch-Str. 6, D-3550 Marburg, F.R.G.

During ontogeny and adulthood neurons are supplied with trophic signals provided by their target cells that ascertain their survival and maintenance of function. It is conceivable but remains to be proven that neurons serving as target cells for other neurons may also store and release neuronotrophic factors (NTFs). We have investigated this issue using a homogeneous population of modified sympathetic neurons, the chromaffin cells from the bovine adrenal medulla, as a putative source of NTFs. Dissociated cell cultures of peripheral and central neurons from embryonic chick and rat and neonatal rat were used as a bioassay system for monitoring the survival promoting effects of the chromaffin cell NTFs. Cell culture media conditioned by purified chromaffin cells, chromaffin cell extracts and the soluble content of chromaffin vesicles all contained NTF activities for a variety of peripheral and central neurons. The NTF(s) from chromaffin vesicles addressed embryonic chick ciliary, dorsal root ganglion, sympathetic and spinal cord neurons, was not inhibited by anti-NGF antibodies and sensitive to trypsin and heat. One Trophic Unit that supported halfmaximal neuronal survival over 24 h amounted to 0.5-10 µg protein depending on the type of neuron tested. Adsorption of the material to polyornithine reduced the activity for ciliary, but not for other ganglion neurons. This suggests the presence of substratum-bound factor(s) with co-trophic effects in the vesicle content. Fractionation of the vesicle proteins on Biogel A 1.5 m revealed that the activity for ciliary neurons had a $M_r$ of 10,000 to 20,000, whereas the activity addressing sensory neurons was in the range of $M_r$ 10,000 to 40,000 with a peak at $M_r$ 20,000 to 30,000. These results suggest that chromaffin cells store and release several proteinaceous NTF activities, which, in vivo, might be relevant to the functional maintenance of autonomic motor and sensory neurons innervating the adrenal medulla.

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313.7 HUMAN NEUROTROPHIC FACTOR: EFFECTS ON MAMMALIAN NEURON SURVIVAL AND NEURITIC OUTGROWTH. Steven T. DeKosky, Stephen W. Scheff, and John Webb*. Depts. of Neurology and Anatomy, Lexington VA and Univ. of Kentucky Medical Centers and Sanders-Brown Research Center on Aging, Lexington, KY 40507

Substances which promote the survival of neural cells in vitro or in vivo, or which promote the outgrowth of neuritic processes in cell culture, have been the target of increasing study over the past several years. The first described neurotrophic factor, nerve growth factor, has both cell survival and neuritic outgrowth inducing properties for a specific subset of central and peripheral nervous system neurons. More recently, factors, usually proteins, have been derived from mammalian brains and shown to promote the survival of neurons in cell culture or in brain tissue transplants. The well characterized factors have been isolated from rodent and other non-human sources.

We report the presence of a stable neurotrophic factor in extracts of normal human brain (HNTF), which promotes survival of mammalian cholinergic neurons in a standardized assay for titration of the presence of such factors. The trophic factor is large (|100 kDal), heat labile, and inactivated by treatment with trypsin. Human cortical tissue preserved for months at -70 C. still contained substantial levels of HNTF. The substance has been found in human cerebral cortex and hippocampus, and also supports survival of fetal rodent hippocampal or striatal neurons in vitro. Under the conditions of this assay, extracts of rat cortex or hippocampus produce cell survival, but do not induce the extensive neuritic outgrowth. The potential role of laminin in inducing the outgrowth seen with HNTF is not yet clear.

If trophic factors play a critical role in nervous system development, repair, or reorganization following trauma, these data indicate that the human central nervous system contains some of the elements critical for this process.

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313.8 PURIFICATION OF A HUMAN RED BLOOD CELL PROTEIN WHICH POSSESSES NEURONOTROPHIC ACTIVITY FOR CULTURED CNS NEURONS: ITS IDENTIFICATION AS CATALASE. P. Walicke, S. Varon and M. Manthorpe. Departments of Biology and Neurosciences, School of Medicine, University of California, San Diego, La Jolla, CA 92093.

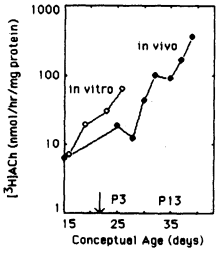
CNS neurons may require specific neuronotrophic factors for their very survival, in analogy to the well documented in vivo and in vitro requirement of sympathetic neurons for Nerve Growth Factor. Recently, we have developed neuronal cell culture bioassays for CNS-directed neuronotrophic agents (Neuroscience 12:33-45, 1984). Using this bioassay, trophic activity has been detected in a variety of conditioned media and tissue extracts. The red blood cell (rbc) was found to be a particularly rich source of a protein exhibiting neuronotrophic activity (Brain Research, 1985, in press).

This protein has now been purified over 1000-fold from human rbc's by ion-exchange chromatography and isoelectric focusing, with a yield of one to two mg of purified material from one unit of aged packed rbc's. The purified protein has been identified as catalase based on its isoelectric point, subunit molecular weight on SDS-PAGE, and ability to degrade hydrogen peroxide. Commercially produced bovine liver catalase, lactoperoxidase, horseradish peroxidase and vitamin E all mimic the ability of the rbc protein to support CNS neuronal survival in vitro. A specific irreversible inhibitor of catalase enzymatic activity, aminotriazole, blocks the trophic activity of both the rbc protein and commercial catalase. Thus peroxidase activity appears to mediate the trophic effects of these proteins. Peroxides, measured by a colorimetric assay, accumulate in the bioassay culture medium in the absence of cells. Removal of this toxic material from the medium may be the basis of the trophic effects of catalase. Supported by NSF Grant BNS 82-18366, ADA Grant 83-10, and a grant from FIDIA Research Laboratories.

313.9 INFLUENCE OF OLFACTORY BULB EXTRACT ON THE SURVIVAL AND DIFFERENTIATION OF CULTURED NEURONS FROM THE BASAL FOREBRAIN. M.P.Lambert and W.L. Klein (SPON: J. Siegel). Dept. of Neurobiology and Physiology, Northwestern University, Evanston, IL 60201.

Embryonic basal forebrain neurons were maintained in cell culture in order to study factors influencing their structural and biochemical development. Presumptive basal forebrain/septal tissue (A.Bjorklund, et al., Acta Physiol. Scan. Supp. 522, 1983) dissected from E16-17 rats and treated with trypsin gave neuronal cell populations that attached well to poly-L-lysine coated plates. Cells maintained in Dulbecco's Modified Eagle Medium (DMEM)/high ascorbate (17.5 mg/l)/ 10% fetal calf serum media showed a time dependent increase in process extension. Processes reached an average length of 214 μ (12x cell body length) by E16/C9. Protein per culture was level up to C8 and then decreased. Cells grown in defined medium showed some neurite development and extension early in culture (C1-2), but lost processes and began shrinking by C3-4. In contrast, cells grown in defined medium containing 25% adult olfactory bulb (OLB) extract showed extensive process development and branching during this period (C1-3). Surprisingly, whole brain extract appeared to inhibit process development as no neurites were observed. These results indicate that OLB, but not other brain regions, from adult rats contain factors that promote neurite outgrowth from embryonic neurons from the basal forebrain. The presence of these factors in OLB is consistent with reports of continuous innervation of the bulb throughout adulthood (A. Cuschieri and L.H. Banninster, J. Anat. 119:277-286, 1975).

The differentiation specifically of cholinergic neurons was determined by measuring Acetyl-CoA: choline O-acetyltransferase, E.C. 2.3.1.6 (ChAT) activity. ChAT activity of cultured cells maintained as above on serum-containing media was compared with ChAT activity of the corresponding brain region in vivo. Results presented in the graph below show that ChAT activity increased in culture from conceptual age 15 to 26 days, comparable to the increase in vivo in the septal region during this period. The greatest increase in ChAT activity in the septal region occurred after P6, and by P17 had reached 90% of adult levels (370 nmol/hr/mg). Future experiments are directed toward measuring levels of ChAT in neurons grown in defined media and assessing the long-term effects of OLB and other extracts on these neurons. (Supported by NIH grant NS18490 to WLK.)




313.10 SOLUBLE STRIATAL EXTRACTS ENHANCE SURVIVAL AND MATURATION OF MESENCEPHALIC DOPAMINERGIC NEURONS IN VITRO. Y. Tomozawa and S.H.Appel, Dept. of Neurology, Baylor College of Medicine, Houston, Texas 77030

Recent studies have suggested that diffusible factors released by neural target tissues enhance survival, growth, and differentiation of neurons within the central, as well as the peripheral nervous system.

Dopaminergic neurons in the substantia nigra project primarily to neuronal targets in the striatum; Prochiantz (Nature, 293, 570, 1981) reported that striatal membranes enhance dopaminergic differentiation in rat mesencephalic cultures.

In the present experiments, soluble extracts from the striatum produce a four fold increase in survival of dopaminergic neurons in mesencephalic cultures prepared from embryonic 14 day old rats, assessed by glyoxylic acid-induced catecholamine histofluorescence. The same soluble extract stimulates high affinity $^{3}$H-dopamine uptake specific for dopaminergic neurons 3.5 to 4.5 fold in the presence of cytosine arabinoside (2μM) in a dose-dependent manner. Such stimulation of high affinity dopamine uptake is significantly reduced following treatment of the soluble extract with trypsin. The striatal extract has no effect on acetylcholine synthesis and choline acetyltransferase in dissociated ventral spinal cord cultures. The soluble factor(s) stimulating survival-differentiation of dopaminergic neurons behave differently from soluble factor(s) stimulating neuronal high affinity GABA uptake on molecular sieving chromatography. NGF, neurotensin and somatostatin have no effect on our bioassay system. The stimulatory effects on dopaminergic neurons are maximal in extracts of the striatum, but are also found in the hippocampus-amygdaloid nucleus area and cerebral cortex, although they are negligible in cerebellum, olfactory bulb, and absent in liver. The effects of soluble striatal extracts on dopaminergic neurons are maximal in mesencephalic cultures from 13 to 16.5 day embryos and drastically decrease thereafter.

313.11 IDENTIFICATION OF A NEUROTROPIC FACTOR PRODUCED BY ASTROCYTES CULTURED FROM RAT CEREBRAL CORTEX. R. Kadle* and R.E. Fellows. Dept. of Physiology and Biophysics, Univ. of Iowa College of Medicine, Iowa City IA 52242.

Survival of dissociated fetal rat brain cells cultured in a serum-free, hormone-free defined medium is enhanced by increasing cell density, suggesting a self-conditioning of medium by tissue specific growth factor(s). At lower densities, neuronal survival can be enhanced by addition of hormones, including insulin, or various established or putative growth factors ranging in size from <1 to >50 kDa. Under serum-free culture conditions, we have observed an absolute requirement for insulin for survival of fetal rat brain cells plated at densities less than 4,000 cells/mm$^2$. At the same time we have been able to grow and maintain CNS neurons plated at much lower densities (<1,000 cells/mm$^2$) in a serum-free defined medium without insulin or other hormones by seeding them on top of an established confluent monolayer of astrocytes. The present study was carried out to determine if the enhancement of neuronal survival might be mediated by a soluble factor or factors secreted by astrocytes into the medium. Astrocyte cultures were prepared from enzymatically dissociated cerebral cortex of 0-1 day old rat pups and grown to confluence in the presence of 15% FBS, after which they were maintained in serum-free defined medium in the absence of insulin. More than 95% of cells in these cultures displayed morphology typical of fibrous astrocytes and positive immunocytochemical staining for glial fibrillary acidic protein. Conditioned medium was collected every 3-5 days and used immediately or stored at -20°. To assay for insulin-like activity, cells dissociated from 17-19 day fetal rat telencephalon (> 80% tetanus-toxin positive) were plated at a density of 3,000 cells/mm$^2$ for determining cell survival and at 8,000 cells/mm$^2$ for measuring $^{3}$H-leucine incorporation. Compared to control cultures grown in defined medium alone, cultures incubated with an ultrafiltration fraction (5 kDa cutoff) of astrocyte conditioned medium showed a significant enhancement of cell survival, similar to that observed in cells incubated with defined medium containing 0.33 uM insulin. Protein synthesis, measured as the incorporation of $^{3}$H-leucine into a TCA insoluble fraction, increased 21% over control when cultures were incubated for 12 hours with the active fraction of astrocyte conditioned medium, compared to 25% over control with 0.33 uM insulin. Neuronal cultures grown in serum-free defined medium also exhibited significant $^{125}$I-insulin binding, in the range of 0.7-1.0 fmol/mg protein. These data suggest that cultured astrocytes elaborate one or more insulin-like factors that may be important for the normal development of CNS neurons.

313.12 A SPECIFIC SEROTONERGIC GROWTH FACTOR FROM 5,7-DHT LESIONED HIPPOCAMPUS: IN VIVO EVIDENCE FROM FETAL TRANSPLANTATION OF RAPHE AND LOCUS COERULEUS NEURONS. F.C. Zhou*, E.C. Azmitia*, S. Auerbach** and B.L. Jacobs**, (*Dept. Biology, NYU, NYC 10003) (**Dept. Psychology, Princeton University, NJ).

Injury induces demonstratable neurotrophic activities in the brain. Specific lesioning of 5-HT fibers from raphe nuclei using 5,7-dihydroxytryptamine (5,7-DHT) (with desipramine pretreatment) in the hippocampal afferent path (cingulum bundle), is able to trigger homotypic sprouting via the other afferent, fornix-fimbria (FF), to reinnervate the hippocampus. This homotypic sprouting of 5-HT fibers is a response to the denervation of 5-HT fibers (Azmitia et al., Nature 274,374(1978); Zhou and Azmitia, Brain Res. 308,53(1984)). The norepinephrine (NE) fibers from locus coeruleus (LC) which travel in the same pathways and terminate in an overlapping area in the hippocampus did not seem to respond to the denervation of 5-HT fibers.

After 5 ug of 5,7-DHT microinjected into the FF (with nomifensine pretreatment), the synaptosomal high affinity uptake (SHAU) of $^{3}$H-5-HT decreased from 137 ± 36 (n=5) in the normal to 50 ± 28 pmol/gm (n=5), and the 5-HT level decreased from 200 + 40 (n=8) to 60 + 40 pg/mg (n=6), while the NE level (170 + 50 pg/mg, n=16) remained similar to the normal (210 + 42 pg/mg, n=16) in the dorsal hippocampus. The growth of the fetal (gestation 14-15 days) 5-HT-raphe and NE-LC neurons were compared when transplanted into the normal versus transplanted into the 5,7-DHT-FF lesioned hippocampus.

Transplants of fetal raphe tissue into the normal dorsal hippocampus produced a hyperinnervation of 5-HT fibers within one month. The SHAU of $^{3}$H-5-HT increased (210 ± 39 pmoles/gm, n=5) 68% above normal level. The concentration of 5-HT (360 ± 140 pg/mg, n=4) rose to 80% above normal. When fetal raphe cells were transplanted into the FF 5,7-DHT lesioned dorsal hippocampus, a dramatic increase in growth of the fetal 5-HT neurons was observed. The SHAU of $^{3}$H-5-HT increased (473 + 182 pmoles/gm, n=5) 345% over intact and 946% over FF-5,7-DHT lesioned hippocampus. The 5-HT concentration increased 255% (510 + 320 pg/mg wt.) over intact and 850% over FF-5,7-DHT lesioned hippocampus.

In contrast, the implanted NE neurons of fetal LC had lower NE levels in the 5-HT partially denervated hippocampus than in normal hippocampus (250 + 50, n=4 versus 420 + 100 pgm/mg, n=3). Thus the 5-HT denervation-triggered trophic signal selectively enhanced the development of the 5-HT neurons but not the NE neurons.

313.13 A SPECIFIC SEROTONERGIC GROWTH FACTOR FROM 5,7-DHT LESIONED HIPPOCAMPUS: IN VITRO EVIDENCE FROM DISSOCIATED CULTURES OF RAPHE AND LOCUS COERULEUS NEURONS. E.C. Azmitia and F.C. Zhou, Dept. of Biology, New York University, New York, NY 10003.

Damage to the CNS triggers a variety of recuperative mechanisms, homotypic (compensatory) collateral sprouting (HCS) is the most specific and beneficial for recovery of function (Azmitia et al., Nature 274,374(1978); Gage et al., Brain Res. 268,271(1983) and Zhou and Azmitia 308,53(1984)). Implicit in HCS is the production by the target area of a trophic factor which stimulates a single group of afferent fibers. We have developed a neurotoxin (5,7-dihydrotryptamine, DHT) injection procedure for eliminating serotonergic fibers afferent to the hippocampus via the cingulum bundle which induces HCS of the undamaged hipp serotonergic fibers entering via the fornix-fimbria (FF). We now present in vivo tissue culture evidence to substantiate the existance of a soluble factor capable of selectively stimulating serotonergic growth.

Mesencephalic raphe neurons from 14 day old rat fetuses were dissociated in EDTA solution by gentle repipetting. The cells at an initial plating density (IPD) between 1.5 - 0.5 X $10^6$ cells/$cm^2$ were grown in 96-well Linbro plates coated with collagen (1 mg/ml) and poly-lysine (25 ugm/ml). Previous studies have shown these cultures contain viable neurons immunoreactive to a specific serotonergic antibody and possess a serotonin high-affinity uptake mechanism (SHAU). The SHAU is linearly related to the (IPD) and is stimulated by dissociated fetal hipp neurons (Azmitia et al., Soc. Neurosci. Abstr., 9,10(1983)). Cultures of dissociated locus coeruleus (LC) neurons were prepared and shown to be immunoreactive to a tyrosine hydroxylase antibody and possess a norepinephrine high-affinity uptake mechanisms (NHAU).

A soluble hippocampal factor was prepared from normal and FF-5,7-DHT (nomifensine pretreated) rats (see Nieto-Sampedro et al., J. Neurosci. 3,2219(1983)). Control aliquots of this preparation were boiled for one hour. All the aliquots were passed through millipore filters (0.2 u pore) before addition (1/50 dilution) to the complete neuronal media (10% FCS in MEM, non-essential aminoacids and 1% glucose).

Addition of the hipp factor stimulated the SHAU in raphe cultures at high IPD (1.5 X $10^6$ cells/$cm^2$) by 57% in three separate experiments after 3 days in culture over that seen when the boiled factor was added. Addition of the 5,7-DHT derived hipp factor stimulated SHAU by 90% in three separate experiments. No stimulation was apparent by either factor at low IPD's. Addition of the hipp factor to LC cultures stimulated the NHAU by 61% in 5 separate experiments over that seen with the boiled factor. In contrast, no apparent stimulation was seen with the factor derived from the 5,7-DHT hipp.

## BRAIN METABOLISM II

314.1 COMPUTERIZED ANALYSIS OF FUNCTIONAL BRAIN IMAGES: APPLICATION TO TOURETTE SYNDROME. G.H. Burrows, D. Bright*, V. Geoffrey* and T. Chase. Experimental Therapeutics Branch, NINCDS, Bethesda, MD 20205; Analytical Chemistry Division, NBS, Gaithersburg, MD 20899.

A computerized procedure for the interpretation of functional images of the human brain has been developed. The method employs LISP, a computer language for symbol manipulation, to drive more conventional image processing routines. Contiguous regions of locally similar functional activity are extracted from the image and used to facilitate the recognition of brain midline, boundaries, and the horizontal level of the image slice. This information is then used to segment the image into roughly homologous regions which can be compared between subjects. To do this a 6x7 grid is separately scaled to each hemisphere in each image yielding rectangular segments roughly 2x2 cm in size. Twenty parameters are calculated from each segment for use in the analysis. This image analysis is performed on a VAX 11/780 computer and a DeAnza 8400 image processor. A large database has been built with over a thousand images and facilities have been completed for accumulating and analyzing data from numerous segment strategies. In addition, LISP is used to write programs for statistical analysis packages.

The procedure has been applied to the study of 12 untreated patients with Tourette syndrome and an equal number of age-matched controls. Each subject was scanned (NINCDS Neuropet tomograph) 30 minutes after receiving 5 mCi of 18-F-fluorodeoxyglucose by intravenous injection. Glucose utilization rates were linearly normalized to the central cerebellum region of each patient. While no consistent difference in overall brain glucose utilization was found, preliminary analysis has shown alterations in certain cortical and subcortical regions mainly at a slice level approximately 84 mm caudal from the vertex. Specifically, glucose values in the region of the frontal and cingulate cortex as well as in the ventral corpus striatum were 17 to 28 percent below control levels ($p < .01$). The clinical severity of motor and vocal tics correlated (Spearmann; $p < .01$) closely with metabolism in these areas. The findings suggest that this computerized procedure may be of use in the analysis of human brain disorders such as Tourette syndrome where no structural abnormalities are available to provide initial localizing information, and where visual inspection of the PET images fails to reveal definite abnormalities.

314.2 CORTICAL AND SUBCORTICAL METABOLIC CORRELATES OF AFFECTIVE ILLNESS USING POSITRON EMISSION TOMOGRAPHY. J.C.Wu1,M.S.Buchsbaum1*,L.DeLisi2*,H.Holcomb2,E.Hazlett1*. 1Brain Imaging Laboratory,UCI,CA 92715,2 NIMH,Bethesda,MD.
Intro:This study extends our previous PET study of affective illness by subdividing bipolar (B) and unipolar (U) depressed subjects and comparing them with normal controls (NC). Methodology: 20 affective (16B,4U,39±12.2yrs,12F,8M) and 24 NC (31±10.4yrs,16M,8F) were studied. Subjects were given 3-5 miCof 18F-2DG while receiving somatosensory stimulation (mild electrical shock to right forearm 1/sec x 30 min)in a darkened and acoustically attenuated room. Subjects were scanned on an ORTEC ECAT II (FWHM=17.5mm). Seven to eight scans parallel to the CM line were obtained. Glucose use was calculated according to the Sokoloff model. Cortical metabolism was measured by peeling off a 2.2 cm cortical rim from the slice. This rim was divided into 4 equal quadrants in each hemisphere. Regions of interest such as the caudate were assessed using an automated template using 3d stereotaxic cooordinates. Results:Affective subjects (B and U) were different from NC in two measures. First, B and U had higher absolute cortical glucose metabolism than NC (NC=19.7±6.3, U=28.6±6.6, B=25.3±8.8; group difference F=4.92,d.f.=2,41,p=.02). Absolute glucose use expressed in micromoles/100g/min. Second, B and U had decreased relative caudate metabolic ratios (NC=1.03±.14, B=0.87±.13, U=0.89±.14,level by structure by group F=2.01,d.f.=3,62,p=.10).

U's were significantly different from B's and NC's on two parameters. First, U's have higher greater absolute antero-posterior gradients than B's or NC's (anteriors greater than posteriors, U=7.0, B=2.2, NC=2.9, group by AP gradient F=7.04, d.f.=3.06,75.07, p=.01). Second, U's have decreased left hemispheric activation relative to the right in absolute terms than B's or NC's (NC=0.8, B=1.2, U=-0.6, group by quadrant by hemisphere F=3.09, d.f.=5.1,95,p=0.012).
Significance: Greater absolute cortical glucose metabolism in affective illness is consistent with hypothesized noradrenergic overactivity. Decreased relative caudate metabolic ratios in affective illness is consistent with decreased dopaminergic activity in affective illness.

314.3 DIFFERENCES BETWEEN ALZHEIMER PATIENTS AND HEALTHY CONTROLS IN PATTERNS OF INTERCORRELATIONS BETWEEN REGIONAL CEREBRAL METABOLIC RATES FOR GLUCOSE. B. Horwitz, C.L. Grady, N.L. Schlageter*, R. Duara and S.I. Rapoport. Laboratory of Neurosciences, National Institute on Aging, National Institutes of Health, Bethesda, MD 20205.

Intercorrelations between resting regional cerebral metabolic rates for glucose (rCMRglc), as determined by positron emission tomography (PET) using [18-F]fluorodeoxyglucose (FDG), provide a measure of the functional associations of different pairs of brain regions (Horwitz et al., J Cereb Blood Flow Metabol 4: 484-499, 1984). The overall set of such correlations allows one to assess integrated activity for the brain as a whole in a specific group of subjects for a particular experimental paradigm. For example, although there is no statistically significant correlation between resting rCMRglc and age (Duara et al., Ann Neurol 16: 702-713, 1984), we found a decrease in the number of statistically significant correlations among parietal lobe regions and between parietal and frontal lobe regions, when comparing 15 healthy men aged 64-83 yr with 15 aged 20-32 yr (Horwitz et al., Soc Neurosci Abstr 10: 1001, 1984).

We report here on the application of this correlation approach to patients with presumed Alzheimer's disease (AD). Patterns of cerebral metabolic correlations in 21 AD patients and 21 healthy age-matched controls in the resting state were compared. On the basis of the Mini-Mental State exam (Folstein et al., J Psychiat Res 12: 189-198, 1975), 10 AD patients were classified as mildly demented (score $\geq$ 21), 8 as moderately demented (20 $\geq$ score $\geq$ 11), and 3 as severely demented (score $\leq$ 10). Cerebral metabolic rates for glucose were determined by PET using [18-F]FDG. Partial correlation coefficients, controlling for whole brain glucose metabolism, were evaluated between pairs of regional glucose metabolic rates in 59 brain regions. Reliable correlation coefficients were obtained by use of the jackknife and bootstrap statistical procedures. Compared with healthy controls, the Alzheimer patients had fewer reliable partial correlation coefficients between frontal and parietal lobe regions. There also was a reduced number of reliable correlations between many bilaterally symmetric brain regions in the Alzheimer's patients, suggesting decreased functional associations between the two cerebral hemispheres in Alzheimer's disease.

314.4 **POSITRON EMISSION TOMOGRAPHY AND PERFORMANCE OF A COGNITIVE TASK.** **I.T. Metz, H.Y. Meltzer, and M. Cooper*. PET Unit--Franklin McLean Institute, Dept. of Radiology, University of Chicago, Chicago, IL 60637.**

**Abnormalities of functioning in frontal cortex have been implicated in some forms of mental illness. We used positron emission tomography to determine whether normal subjects and patients with severe psychiatric illness differ in their regional cerebral metabolism of $^{18}$fluoro-deoxyglucose (FDG). During the uptake period of the FDG, subjects performed a variant of the Wisconsin Card Sorting Test (WCST), a task which has been shown to be related to functioning of the frontal lobes.**

**Seven normal subjects, six RDC schizophrenic patients, and four non-schizophrenic patients were studied. All patients were hospitalized at the Illinois State Psychiatric Institute and had been unmedicated for at least ten days at the time of testing. After preliminary positioning and transmission scans in the PETT VI scanner, subjects were injected with four to ten millicuries of FDG via IV catheter; subjects then performed the WCST for 40 minutes prior to being repositioned and scanned for 30 minutes. For 28 brain regions, we determined activity as a ratio of counts in that region to the average counts for all regions.**

**Both patient groups showed a tendency toward slightly less metabolic activity than the normals in all cortical regions. There was no evidence, however, for a specific effect in frontal regions. Overall, the ratio of frontal to non-frontal cortical activity was virtually identical in the three groups (0.91, 0.90, and 0.92 respectively for normals, schizophrenics, and non-schizophrenics). All three groups also showed a tendency toward higher relative frontal activity on the right than on the left side. Relative metabolic activity in the schizophrenic group was significantly higher than in the normals in basal ganglia regions on both sides.**

**As expected, both patient groups performed more poorly than the normals on the WCST: "conceptual level responding" scores were 56%, 35%, and 40%, while "perseverative errors" were 14%, 16%, and 29% for normals, schizophrenics, and non-schizophrenics, respectively. Across all subjects, there was a high correlation between relative metabolic activity in the right parietal cortex and perseverative error percentage ($r = 0.58$): higher parietal metabolism was associated with higher error rates. No other relationship was found between task performance and a brain region. This relationship may be spuriously due to the large number of statistical tests, or it could reflect underlying anatomical relationships between parietal and frontal cortical regions.**

**These results do not support the hypothesis of a frontal cortex abnormality in psychiatric patients. This failure to support the hypothesis may be due to differences in patient samples between this and other studies. It may also be due to differences in the task conditions: when patients are activated in a manner similar to normal subjects, differences which appear in "resting" conditions may disappear.**

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314.5 BRAIN PATTERN SPACE: A NEW ANALYTIC METHOD UNCOVERS COVARYING REGIONAL VALUES IN PET MEASURED PATTERNS OF HUMAN BRAIN ACTIVITY. J.R.Moeller*, B.T.Volpe*, J.S.Perlmutter*, M.E.Raichle, M.S.Gazzaniga. Cornell Univ. Med. Col., NY, NY 10021; Washington Univ. Sch. of Med., St. Louis, MO 63110.

One of the main goals of PET image analysis has been to demonstrate that different clinical populations are discriminable on the basis of the differences in their images of mean regional values of PET measured brain activity. At the same time, a major limitation has been that this kind of analysis does not provide a direct means of determining the constituent patterns of covarying regional values that actually produced the differences among population images. By way of example: consider a comparison of a sample of normal subjects with a sample of subjects exhibiting a common pathology in which six regional differences occurred out of forty regions measured. Moreover, suppose that two distinct pathological processes contributed to the pattern of differences: one process affected the first four regions and the second, the last four of the six affected regions. The analysis described above would not reveal this important biological fact. To date, the research strategy employed to resolve this indeterminacy of PET image analysis has been to account for the changes created by a clinical pathology in terms of changes in PET measured brain activity that can be effected by altering specific biological, sensory, and/or cognitive processes. But the adverse side of this methodology is that the scientific value of such PET studies is considerably diminished in the event that the hypothesized association between functional dissociations and the differences in population images is subsequently refuted.

The aim of the present work is to demonstrate a new analytic method which recovers directly from PET data, patterns of covarying regional activity which represent important biological and computational constraints on human brain functioning. The demonstration is based on the PET data from a sample of 25 Normal individuals and a pathological population of 11 Amnestics, in which a subject's brain pattern consists of 26 regional values, measured while the subject is in an alert resting state. The brain patterns of subjects of both populations are plotted as individual points in a 26 dimensional Brain Pattern Space. Discriminate Analysis indicates that these two populations are perfectly discriminable in Brain Pattern Space--even though the mean patterns for the two populations are correlated at the .92 level. Thus, what differentiates these two populations are differences in the way that the brain patterns of different subjects systematically vary within each population.

The main finding is that the between-population differences in covariance are uniquely attributable to the different patterns of covariance exhibited by a specific set of three regions. That is, while there are other sets of regions which exhibit similar patterns of covariance in both the Normals and Amnestics, the different patterns of covariance produced by these three regions account for essentially all the between-population differences that can be reliably estimated from the present two clinical samples. The biological interpretation is that a particular pattern of normal regional covariance has been transformed by the pathology of the Amnestics. The three covarying regions are nominally the caudate region, anterior cingulate region, and medial basal frontal lobe. At a functional level, these data underscore the importance of considering the role attentional mechanisms play in the production of memory disorders. (Aided by NS 033426.)

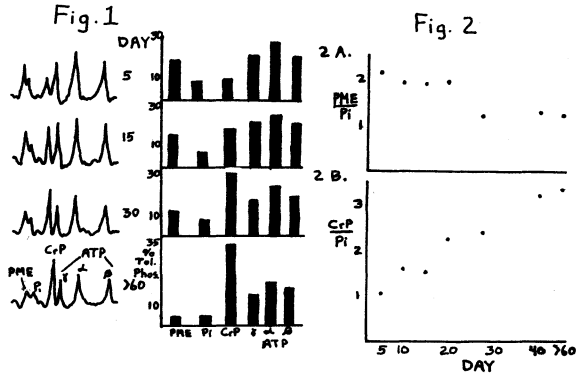
314.6 EFFECT OF SCAN LENGTH ON THE MEASUREMENT OF LOCAL CEREBRAL BLOOD FLOW WITH EMISSION TOMOGRAPHY AND THE CLASSIC AUTORADIOGRAPHIC TECHNIQUE P. Herscovitch* and M.E. Raichle (SPON: D.B. Clifford). Washington University School of Medicine, St. Louis, MO 63110

Several methods for measuring local cerebral blood flow (CBF) with emission tomography are based on Kety's model describing the behaviour of flow-limited tracers. These methods are analogous to the tissue autoradiographic or sampling method (Kety, Methods Med Res 8:228) but use tissue count data obtained in vivo during either a single scan or multiple sequential scans. Eklof (Acta Physiol Scand 91:1) studied the Kety tissue sampling method in rats and found that measured CBF progressively underestimated the true flow as tracer infusion time was increased. This effect was attributed to diffusion limitation of the radiotracers studied. Because this error was appreciable, we elected to study, in humans, the effect of scan length on CBF quantitation in a positron emission tomographic adaptation of the tissue autoradiographic method (Herscovitch et al, J Nucl Med 24:782). Both a radiotracer with a modest diffusion limitation, O-15 water, and a highly diffusible tracer, C-11 butanol, were studied in each of our subjects. The PETT VI tomograph was used to collect cerebral count data in sequential 40 sec time frames following intravenous injection of radiotracer. Frames were combined to calculate CBF for effective scan lengths of 40, 80, 120, and 160 sec. Increasing scan length resulted in average CBF measured with O-15 water decreasing from the 40 sec measurement by an average of 20% at 80 sec, 27% at 120 sec, and 31% at 160 sec. This decline occurred at high as well as lower flows at which water is freely permeable. Furthermore, similar declines in measured flow occurred with the diffusible tracer, C-11 butanol. Simulation studies were used to seek possible explanations for these flow declines. It was found that underestimations of flow that increase with experiment length would result from local tissue heterogeneity, systematic underestimation of tissue activity, or considerable lag in the sampled peripheral arterial time-activity curve with respect to the true input function to the brain. However, these effects could not easily explain the degree of observed time dependence of flow measurement.

In summary, not only the tissue autoradiographic method but also its adaptation to emission tomography yields declining flow values with increasing time. As this phenomenon occurred with a freely diffusible tracer, C-11 butanol, as well as with O-15 water, assumptions in the Kety model other than tracer permeability must be involved.

314.7 PHOSPHORUS-31 NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY OF IN VIVO RAT BRAIN: DEVELOPMENTAL CHANGES. K.H. Taber, J.J. Ford* and R.N. Bryan. Dept. of Radiology - Magnetic Resonance Center, Baylor College of Medicine, Houston, TX 77030.

Phosphorus-31 nuclear magnetic resonance spectroscopy was used to compare the relative concentrations of the phosphorus containing compounds phosphomonoester (PME), inorganic phosphate (Pi), creatine phosphate (CrP) and adenosine triphosphate (ATP) as well as intracellular pH in vivo in neonatal and adult rat brain. Differences were found between neonate and adults in the ratios of CrP and PME to Pi and ATP (Fig. 1). The relative intensity of the CrP peak increased and the PME peak decreased with increasing age. The CrP increase was gradual from 5 days of age to adulthood, a change which is partially accounted for by the concomittant decrease in the T1 relaxation time for CrP. In addition, however, it is extremely likely that the actual tissue concentration of CrP increased during this period. The decrease in the PME peak, on the other hand, occurred rather abruptly between 20-30 days of age. The T1 of PME was also decreasing during this period of time, suggesting that the true change in in PME relative to the remainder of the P-31 spectrum was greater than was apparent from these measurements. Maturational curves for these measures are presented (Fig. 2). The relative size of the ATP and Pi peaks and the calculated intracellular pH did not change with age, although it is likely that the absolute level of both Pi and ATP increased from neonate to adult.




314.8 DETECTION OF $^{13}$C-GLUCOSE INCORPORATION INTO GLUTAMATE AND LACTATE BY MAGNETIC RESONANCE SPECTROSCOPY (MRS) IN RAT BRAIN . G. A. Rosenberg, S. Chavez, and J. R. Brainard. Dept. of Neurology, Univ. of New Mexico Sch. of Med., Albuquerque, NM 87131, and Veterans Administration Medical Center, Albuquerque, NM 87108, and Los Alamos National Laboratory, Los Alamos, NM 87545.

Glucose utilization is essential for brain function. Attempts to follow glucose metabolism are complicated by its multiple metabolic products. Magnetic resonance spectroscopy (MRS) is an insensitive method with a high degree of molecular specificity. In order to determine the potential usefulness of MRS to study glucose metabolism in brain, we followed the metabolism of $^{13}$C-glucose in rats. Adult rats (200-400gms) were anesthetized with an intraperitoneal injection of pentobarbital (50mg/kg). Forty micromoles of $^{13}$C-glucose dissolved in saline was injected as a bolus into the femoral vein. Samples of arterial blood were collected at 5 to 15 minute intervals. After 5, 15, 30 and 60 minutes groups of 2 animals each were sacrificed by funnel freezing the brain with liquid nitrogen. The brain tissue was extracted with perchloric acid under cold conditions. The extracts were analyzed by MRS with a 7 Tesla high resolution spectrometer. We found signals from glutamate in the 5, 15 and 30 minute samples. The label from the enriched glucose was found in the 2,3 and 4 positions. Doubly labelled glutamate was not detected. Lactate was detectable in the samples. The labelling pattern of glutamate suggests that the labelled glucose carbon passed through the Krebs cycle only 1 or 2 times. This low level of activity may have been due to the anesthesia or that the levels of doubly labelled gluatamate were too low to detect. These preliminary studies demonstrate the potential for MRS to follow metabolic pathways in the brain.

314.9 LOW-COST, COMPUTER ASSISTED DENSITOMETRY FOR AUTORADIOGRAPHY: A PRACTICAL GUIDE TO SYSTEM COMPONENTS. P. Ramm, Dept. of Psychology, Trent University, Peterborough, Ont. K9J 7B8

Recently, imaging boards which plug directly into the IBM PC have become available. With such a board, a solid state scanner and commercially available software, it is possible to build an accurate densitometry system, using only off-the-shelf components, for less than $12,000.

Our system consists of a COMPAQ Deskpro personal computer, equipped with 640K of RAM, an 8087 chip, and an external hard disk and tape backup unit. The COMPAQ is highly compatible with the IBM PC, but runs 2-3 times as fast. The imaging component is an Imaging Technology (Woburn,MA) PCVISION frame grabber, which plugs into the COMPAQ motherboard. The PCVISION captures an input video signal 30 times/sec, and simultaneously displays and stores the image in a 512x512x8 bit (256 gray level/pixel) array. The image can be displayed in black and white or in pseudocolor, on an Electrohome ECM 1301 high resolution color monitor. Image contrast or color coding can be easily manipulated. Image data are read using "sample windows", apertures of any desired size which are placed over brain regions under mouse control. A file creation protocol stores data (optic density values) under animal, section, structure and sample number heading. Other routines control "visuals" (contrast, etc.), convert optic density values to tissue equivalent uCi/g or levels of LCGU, perform data normalization, summary statistics, and format the data into a file easily read by the operator or by a statistics package.

The imaging system receives input from an RCA TC2800 solid state CCD camera, mounted on a sturdy copy stand. The camera provides an array of 403Hx512V pixels at a signal to noise ratio of better than 59 dB. Autoradiographs are illuminated on a light box, which contains a fluorescent lamp for visual inspection, and an incandescent spot illuminator for precision imaging. Light output of the spot illuminator can be varied under control of a highly-stable DC power supply.

Total hardware cost of the above system in the US is about $12,000 without the computer, $14-16,000 with. This includes the imaging board, 25 megabyte hard disk and tape unit, camera, copy stand, and precision light source. A simpler system, without a hard disk/tape for example, costs about $12,000 with computer. A proficient programmer could construct useable software within about 100-300 hours, or a mature software package with all necessary hardware specifications and light source construction instructions can be purchased for about $2000.

314.10 MODELING OF LOCAL CEREBRAL GLUCOSE METABOLISM BY $^{19}$F FDG NMR ONE DIMENSIONAL ZEUGMATOGRAPHY. T. Nakada, I. L. Kwee*, and C. B. Conboy*. Neurochem. Res. Lab., VA Med. Ctr., Martinez, CA 94553 and Dept. of Neurology and NMR Facility, Univ. of California, Davis, CA 95616.

We have previously shown that contrary to the concept of the limited nature of 2-fluoro-2-deoxy-D-glucose (FDG) metabolism in vivo, the metabolism of FDG in brain extends beyond the hexokinase reaction and that this metabolism can be monitored non-invasively in vivo using $^{19}$F nuclear magnetic resonance (NMR) spectroscopy (Nakada et al, Fed Proc 44:608, 1985, Kwee et al, Fed Proc 44:609, 1985). Applying the rotating frame zeugmatographic technique, we studied regional metabolism of FDG in vivo in rat brain. FDG (400mg/kg) was injected intraperitoneally in young adult Sprague-Dawley rats (150-250g). To obtain stable contents of each metabolite, the animal was sacrificed by overdose of pentobarbital just prior to each study. The animal was subsequently placed in the NMR probe which contained a one turn oval (2.5 x 3.5cm) surface coil centered over the rat calvarium. After shimming on tissue water hydrogen (proton line width 50Hz), $^{19}$F signals were obtained using a spectrometer operating at 4.69T (Nicolet NMR System NT-200) at 188.2 MHz. Sets of free induction decays (FIDs) were obtained with consecutively incremented RF pulse widths. Two dimensional Fourier transformation of data sets provided sets of spectra representing local contents of FDG and its metabolites. The present study indicates the feasibility of establishing a model of regional glucose metabolism by $^{19}$F NMR spectroscopy.

This work was supported by UCD grants D-1783 (TN), NMR-8540 (TN), and D-1921 (ILK).

314.11 **AUTORADIOGRAPHIC DOUBLE ISOTOPE MEASUREMENT OF LOCAL CEREBRAL GLUCOSE METABOLIC RATE (lCMRG) AND ACID-BASE STATUS (ABS) IN RAT BRAIN.** K. E. Peek, A. H. Lockwood, M. Berridge*, L. Bogue*. University of Texas Health Science Center at Houston, Houston, TX

Data suggest that high glycolysis rates in ischemic brain generate excess lactate resulting in acidosis, a factor that may determine the post-ischemic recovery potential. We developed a double isotope autoradiographic method to measure simultaneously lCMRG and ABS or "pH" in rat brain by exploiting the difference between room temperature vapor pressures of [2-$^{14}$C]-2-deoxy-D-glucose (DG) and its metabolite DG-6-$PO_4$, and 5,5-dimethyl [2-$^{14}$C] oxazolidine-2,4-dione (DMO) (pK 6.13). The separation of the tracer molecules in 20-micron brain sections and plasma form the crux of the method: solvent extraction with methylene chloride (MC), ethyl acetate (EA), and chloroform (C), as well as sublimation were evaluated. Extraction of brain sections yielded:

| | DG + DG-6-$PO_4$ residual (%) | | | DMO residual (%) | | |
|---|---|---|---|---|---|---|
| | 1 h | 1.5 h | 48 h | 1 h | 1.5 h | 48 h |
| EA | 95±12 | | 78±20 | 34±0 | 29±3 | ND |
| MC | 84±6 | | 106±13 | 11±.1 | 13±3 | 8.7±.1 |
| C | 120±12 | 105±12 | 85±8 | 9.4±0 | ND | ND |

± SD, ND = not detected, 1 h C, 48 h EA and C DG, and all DMO ≠ 100%, P<0.01. Residual $^{14}$C after 14-day sublimation at room temperature was 99±3% and 3.0±1.5% for DG and DMO brain sections respectively. Apparent increases in residual $^{14}$C levels after solvent extraction are attributable to a reduction in tissue volume and beta self-absorption after brain lipid removal and would require inclusion of a correction factor in calculations. Therefore, we have chosen sublimation for isotopic separation. Rats are injected iv with 350 uCi/kg DMO and 120 uCi/kg DG, and serial blood plasma samples are obtained for tracer content measurements. Injections are made sequentially, DMO then DG after DMO equilibration permitting extrapolation of steady-state DMO levels after the DG injection; alternatively, isotopes are injected simultaneously and TLC separation of tracers determines plasma levels. Brain slices are cut in a cryostat and exposed to film along with standards to yield a DG + DMO image. After sublimation, a second exposure is made to yield a DG image. Autoradiograms are imaged with computer-controlled Eikonix digital camera. After conversion to isotope content (nCi/g), image processing subtraction techniques allow generation of DG and DMO images of the same section of tissue. Computation of lCMRG and ABS follow, using standard equations. We hypothesize that regions of increased lCMRG around ischemic regions of brain are quantitatively linked to measurable increases in acidosis.

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314.12 COMPARISON OF THE TOPOGRAPHIC DISTRIBUTION OF RADIOACTIVITY IN THE HIPPOCAMPAL FORMATION AFTER INJECTION OF (1-$^{14}$C)-GLUCOSE OR ($^{14}$C)-2-DEOXYGLUCOSE. W.E. Stumpf and G.E. Duncan*, Dept. of Anatomy, Univ. of NC at Chapel Hill, Chapel Hill, NC 27514.

After intravenous injection of (1-$^{14}$C)-glucose or ($^{14}$C)-2-deoxyglucose, the relative uptake and retention of radioactivity in different hippocampal subregions was compared. For quantitation of radioactivity, autoradiograms were prepared by thaw-mounting 4um frozen sections on nuclear emulsion-coated slides. Silver grain densities in autoradiograms were evaluated with a computer-assisted video system that allows automated counting of silver grains. Striking differences were found in the hippocampus between ($^{14}$C)-2-deoxyglucose autoradiograms and ($^{14}$C)-glucose autoradiograms (see table below). The CA-3 pyramidal cell field retained considerably more radioactivity than other pyramidal cell fields after injection of ($^{14}$C)-2-deoxyglucose, while after injection of (1-$^{14}$C)-glucose, the retention of radioactivity was similar in all pyramidal cell fields. After (1-$^{14}$C)-glucose injection, the dentate gyrus contained relatively high levels of radioactivity and more $^{14}$C accumulated in the granular layer compared to the molecular layer. After ($^{14}$C)-2-deoxyglucose injection, there was uniformly less radioactivity throughout the dentate gyrus when compared to rats injected with (1-$^{14}$C)-glucose and there was no preferential accumulation of ($^{14}$C)-2-deoxyglucose in the granular layer compared to the molecular layer.

*Table: Silver grain densities in different hippocampal regions after injection of ($^{14}$C)-2-DG or (1-$^{14}$C)-glucose*

| | Silver grains/1000um$^2$ | |
|---|---|---|
| Region | ($^{14}$C)-2-DG | (1-$^{14}$C)-glucose |
| Dentate molecular | 24.9±1.9 | 33.0±4.4* |
| Dentate granular | 23.9±1.7 | 38.5±4.2* |
| CA-1 stratum radiatum | 24.1±1.8 | 33.9±4.2* |
| CA-1 pyramidal | 30.9±3.8 | 41.5±4.2* |
| CA-3 stratum radiatum | 25.7±3.9 | 29.1±3.1 |
| CA-3 pyramidal | 39.0±3.4 | 39.1±4.3 |

Rats were injected i.v. with ($^{14}$C)-2-DG or (1$^{14}$C)-glucose and killed 30 min after the injections. Data are expressed as mean± SEM. * p<.01 compared to ($^{14}$C)-2-DG

These results imply that, in the hippocampal formation, different cellular functions are assessed by determination of the uptake and retention of radioactivity from (1-$^{14}$C)-glucose and ($^{14}$C)-2-deoxyglucose.

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314.13 HIGH RATE OF GLUCOSE UTILIZATION IN THE FASCICULUS RETROFLEXUS IN THE BRAIN OF THE CONSCIOUS MOUSE. T. Jay*, F.R. Domer and L. Sokoloff. Lab. Cerebral Metabolism, NIMH, Bethesda, MD 20205

In a previous study the fasciculus retroflexus, a white matter tract, was found to have an unusually high rate of glucose metabolism, 77 to 96 μmoles/100 g/min (Jay, Jouvet and Des Rosiers, Brain Res., in press). Histochemical examination revealed no higher activity of cytochrome oxidase in this tract. Pulsinelli and Duffy (Science, 204:626, 1979) reported that hypoxia increased several-fold the rate of glucose metabolism in white matter. As it was possible that anaerobic glycolysis was responsible for the unusually high rate of glucose utilization, we have therefore attempted to alter the oxygen supply of the fasciculus retroflexus in the mouse. Cannulae were placed in a femoral artery and vein of C57BL6 mice 24 hours prior to the experiments employing deoxyglucose (DG). The experiments involved the exposure of the mouse either to room air, 100% oxygen, or 10% carbon dioxide in 90% oxygen. 15 min later, [$^{14}$C]DG (125 μCi/kg) was administered intravenously and timed blood samples were taken during the next 45 minutes. The animal was killed with pentobarbital intravenously and the brain quickly dissected and frozen for subsequent sectioning and autoradiography. The glucose utilization was calculated from the arterial plasma curve and quantitative densitometry.

| | Air | $O_2$ (100%) | $O_2$ (90%) + $CO_2$ (10%) |
|---|---|---|---|
| Fasciculus retroflexus | 97 ± 4 | 77 ± 6* | 56 ± 3*** |
| Corpus callosum | 35 ± 2 | 28 ± 3* | 21 ± 3** |
| Mamillary body | 98 ± 9 | 78 ± 9 | 60 ± 5* |

The values represent the mean local cerebral glucose utilization (μmoles/100 g/min) ± S.E.M. in four animals per group.
*p < 0.05 **p < 0.01 ***p < 0.001

Significant decreases in the rate of glucose utilization in the fasciculus retroflexus occurred in the animals exposed to oxygen or oxygen and carbon dioxide, with the decrease being more significant with the combination of gases. Similar significant decreases, though smaller, were found in the corpus callosum. A significant decrease occurs only with the gas mixture in the mamillary body, a gray matter area. The high blood flow and high oxygen tension associated with the gas mixture provided the necessary oxygen to decrease the rate of glucose utilization in all of the structures. Still the decreased rate in the fasciculus retroflexus to the level of white matter found normally had not occurred. Therefore, hypoxia does not explain the high normal glucose utilization of this structure.

314.14 THE LOCUS COERULEUS MODULATES CEREBRAL GLUCOSE UTILIZATION DURING AUDITORY STRESS - A. Justice, S. M. Feldman, and L. L. Brown. Dept. of Psychol. New York Univ., New York, NY 10003.

Hypotheses about the function of the locus coeruleus (LC) have included a role in modulation of cerebral metabolism, particularly during periods of increased metabolic demand. Our earlier experiment (Soc. Neurosci. Abs., 10: 1003, 1984) examined the effects of bilateral 6-hydroxydopamine (6-OHDA) lesions of the LC on local cerebral glucose utilization (LCGU) in rats "at rest." We now report that lesions in animals subjected to stressful auditory stimulation have different effects on LCGU than do lesions in animals in a quiet environment.

The LC was infused bilaterally with 6-OHDA (8 μg) or with its vehicle (2 μl saline). Two weeks later animals were processed for determination of LCGU by the quantitative 2-deoxyglucose (2DG) method of Sokoloff. During the terminal experiment white noise was used to increase metabolic demand. An auditory stimulus was chosen because it is a natural stimulus that has widespread effects on the brain. Sound intensity was set at a stressful level (95 db) because the LC has been shown to respond to stressful stimuli; a behavioral assay in different animals confirmed that the stimulus was stressful under the temporal conditions of the 2DG experiment (Justice and Maone, unpublished observations). Lesions were verified by LC cell counts and by fluorescence microscopy.

Two by two analyses of variance (lesion/intact, noise/silence), combining the data of the present experiment with the earlier one, revealed a significant interaction effect for 37 of 109 structures measured, as well as for the average LCGU of all 109 structures (an estimate of whole brain glucose utilization). Simple effects analysis revealed the source of the interactions: Lesions had no significant effects on LCGU in animals in silence (7% lower average for all structures in lesioned animals); by contrast, lesions in animals subjected to noise caused increases in LCGU in 47 structures (18% higher average). Alternatively, noise had less effect on intact than on lesioned animals: 30 vs. 106 structures measured showed significant simple effects (or a 25% vs. 58% increase in average glucose utilization for intact and lesioned, respectively).

We conclude that norepinephrine (NE) from the LC acts to attenuate the metabolic increases caused by intense auditory stimulation. Our results are consistent with findings that NE is released during stress and that it can act as an inhibitory neurotransmitter. Metabolic inhibition during large and widespread increases in activity may also be related to findings that LC-derived NE has an inhibitory effect on induced seizure activity.

314.15 DEMONSTRATION OF INTERDIGITATING FUNCTIONAL COLUMNS IN RHESUS MONKEY USING A DOUBLE-LABEL 2-DG SUBTRACTION TECHNIQUE.
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We have previously described a double-label version of the 2-DG technique which allows comparison of brain metabolism associated with two distinct conditions within a single subject (Friedman et al., Neurosci. Abstr., 10: 1002, '84). Briefly, $^{14}$C-2DG and then $^{3}$H-2DG were sequentially administered prior to each of two distinct test conditions in the same animal. X-ray and Ultrofilm images of each brain tissue section were made and a computer analysis was devised which digitized the films, converted each image to radioactivity levels, and produced separate images of $^{14}$C and $^{3}$H activity for each section by aligning the two images and then using a subtraction algorithm.

In our earlier work, we validated this double-label 2-DG method by sequentially giving sensory or electrical stimulation restricted to one hemisphere and then the other and finding that the appropriate label predominated in each hemisphere. In the present study the ocular dominance columns of primate visual cortex were stimulated to determine if the technique is also capable of revealing differential patterns of metabolic activity at the level of single columns within a single brain structure.

The monkey viewed drifting square-wave gratings whose orientation changed every 10 seconds in order to stimulate most cells. The left eye was occluded before the $^{14}$C-2DG injection and 30 minutes later the occluder was moved to the right eye and $^{3}$H-2DG was injected. After another 30 minutes the monkey was killed with barbiturate and perfused. Coronal sections (20um) of the occipital lobe were exposed on both films and analyzed to yield separate $^{3}$H and $^{14}$C images of the same section.

In striate cortex (V1) both images had bands of high metabolic activity, roughly 0.5mm wide and most intense in layer IV. The two patterns of label were predominantly interdigitated in that high metabolic activity of one label coincided with low metabolic activity of the other label and vice versa. However, some bands in V1 were coincident in the paired $^{3}$H and $^{14}$C images. Metabolic labeling in V2 was very different: bands of high activity were wider than in V1 and appeared to always be coincident in both labels. We hypothesize that these coincident zones in both V1 and V2 might be aligned with the cytochrome oxidase intense blobs and bands of layer III that respectively mark zones of chronically high metabolic activity in these areas. We conclude that the double-label 2-DG technique can reveal differential functional patterns of metabolic activity within single brain structures and can differentiate between acute, task-specific and chronic, nonspecific metabolic activity. Supported by EY04740, MH38546, and NS19610.

314.16 LOCAL GLUCOSE METABOLIC RATES IN PRIMATE THALAMUS DURING RECOVERY FROM UNILATERAL ABLATION OF CEREBRAL CORTICAL AREAS 4 & 6.
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In humans and infra-human primates, a lesion of motor cortex results in an immediate contralateral hypotonic hemiplegia. With time the hemiplegia becomes hypertonic and some recovery of function occurs. Little is known about the central nervous system structures and events responsible for the recovery process. We present data on glucose utilization in the thalamus of animals undergoing recovery from ablation of the motor cortex.

M. fascicularis monkeys (2.5-3.5 Kg) were used. Three groups of 6 animals each include: 1) an unoperated control group; 2) animals studied 1 week after unilateral (left-sided) ablation of cerebral cortical areas 4 & 6; and 3) animals studied 8 weeks after unilateral ablation of areas 4 & 6. The ablations were performed with subpial resection using pentobarbital anesthesia and sterile procedures. The deoxyglucose technique was used to evaluate local cerebral metabolic rate for glucose (LCMRG)(Sokoloff et al, 1977). LCMRG was determined bilaterally for 9 thalamic nuclei: anterior thalamus (AT); centrum median (CM); lateral geniculate (LG); medial geniculate (MG); medial dorsal (MD); pulvinar (PUL); ventral anterior (VA); ventral lateral (VL); ventral posterior (VP). The boundaries of nuclei were determined microscopically from adjacent Nissl stained serial sections. Each nucleus was analyzed as a whole, i.e. not broken down into its various subdivisions. The Wilcoxon Signed Ranks test was used to evaluate differences in the same structures on the two sides of the brain in each group.

In general, for structures with significant left vs right differences, both sides had decreased LCMRG at 1 week, and the left side was reduced substantially over the right. At 8 weeks there was recovery towards control levels for both sides, but the left remained decreased compared to the right. At 1 week the left LCMRG was significantly less than the right in MD, CM, VP, VL, VA, & PUL. At 8 weeks the left LCMRG was significantly less than the right in MD, CM, AT, VP, VL & VA. There were no significant left vs right differences in MG or LG at 1 or 8 weeks after ablation.

The findings indicate a reduction of LCMRG in some thalamic structures receiving direct input from the ablated areas of cerebral cortex (VA, VL, VP, CM, MD) and in some structures without direct projections (PUL, AT). The latter structures are affected over at least one intervening synaptic connection. Some recovery of LCMRG occurs with time in all affected structures, but the degree varies widely. The recovery of LCMRG may be associated with functional recovery in the animal.

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314.17 REGIONAL CEREBRAL BLOOD FLOW IN THE AWAKE, UNSTRESSED RAT.
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Measurement of cerebral blood flow (CBF) usually requires invasive sampling of arterial blood. As a result, CBF measurements have been made using anesthetized or awake-restrained laboratory animals. CBF may be altered by anesthetics, artificial respiration or the stress produced by restraint. Therefore, we developed a method that allows measurement of regional CBF (rCBF) in awake, unstressed, freely-moving rats. Arterial and venous catheters were placed using barbituate anesthesia and routed subcutaneously to exit near the skull. Rats were placed in individual enclosures, and a swivelling tether fixed to the skull guided the catheters outside the enclosure. A recovery period of 5 to 7 days was allowed prior to rCBF measurement. At that time each rat was (1) free of anesthesia, (2) recovered from surgery, (3) isolated from the investigators, (4) in a familiar environment, and (5) able to move about freely. rCBF was measured using [$^{14}$C]-iodoantipyrine. The time delay and dispersion of the arterial tracer concentration profile induced by the long catheter was corrected using a numeric solution of the convolution integral modeled for the catheter. Physiologic parameters measured immediately prior to rCBF measurement indicated that the rats were not stressed:

| | |
|---|---|
| Plasma glucose | 6.26 um/ml |
| Plasma epinephrine | 47 pg/ml |
| Plasma norepinephrine | 182 pg/ml |
| Heart rate | 353 bpm |
| Mean arterial blood pressure | 93 mmHg |
| $PaCO_2$ | 40.6 mmHg |
| $PaO_2$ | 90 mmHg |
| pHa | 7.416 |

rCBF values measured in freely-moving rats were higher than those reported by most investigators but not all. It is difficult to make detailed comparisons because physiologic parameters important to CBF regulation such as $PaCO_2$ and plasma epinephrine were not reported in most studies. However, when regional cerebral glucose utilization (measured using freely-moving rat model) was plotted as a function of CBF for the corresponding region, the relationship was described by a straight line with a slope of 0.49 (r=0.99). This slope represents the arterio-venous difference of glucose across the brain and is consistent with arterio-venous differences of glucose that have been directly measured. This model allows rCBF to be measured without anesthesia or restraint and offers a method of mimicing the natural physiologic state. This work was supported in part by grants from the American Heart Association (83-903 83-906 84-122) and the PHS (NS15926 NS19341).

314.18 REGIONAL GLUCOSE AND β HYDROXYBUTYRATE USE BY DEVELOPING RAT BRAIN
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Rates of glucose and D-β-hydroxybutyrate (D-β HB) use were determined in five brain regions of 20-day-old rats. The regions studied were cerebral cortex, thalamus, striatum, cerebellum, and brainstem. The tracers for determining rates of substrate use were [$^{3}$H] fluorodeoxyglucose (FDG) and [3-$^{14}$C]-D-β HB. The animals were sacrificed in a 6 kW, 2450 MHz focused microwave apparatus (Medina and Stavinoha, Brain Res., 132:149, 1977) at two and five minutes after intravenous isotope administration. Radioactivity in neutralized perchloric acid extracts of brain was chromatographically separated into its [$^{3}$H] FDG and [$^{3}$H] FDG-phosphate components and the rate of glucose use calculated as previously described (Miller and Corddry, J. Neurochem., 35:1202, 1981), using 0.6 as the rate of phosphorylation of FDG relative to glucose. Radioactivity in brain metabolites of [3-$^{14}$C]-D-β HB was calculated as the difference between total $^{14}$C in the extract and $^{14}$C in D-β HB in the extract. The latter was estimated from the product of brain D-β HB content (nmol/mg protein) and blood D-β HB specific activity (dpm/nmol). Blood and brain D-β HB specific activities were assumed to be equal to one another. Rates of D-β HB use were then calculated in the same fashion as glucose use rates. All animals were fed ad lib. Eight minutes prior to isotope administration they were injected intraperitoneally with normal saline or DL-β HB (10 mmol/kg).

Blood D-β HB levels averaged 0.21 μmol/ml in saline-injected and 3.13 μmol/ml in hyperketonemic rats. Rates of glucose utilization were significantly heterogeneous between regions in both groups: thalamus > cerebral cortex > striatum > brainstem > cerebellum. Rates of D-β HB use varied significantly between regions only in the saline group, with the brainstem rate being significantly lower than other regions. Regional rates of D-β HB use did not correlate with regional rates of glucose use in either group. Whole brain glucose utilization is lower in hyperketonemic 20-day-old rats (Miller et al., Dev. Brain Res., 4:443, 1982) and, in this study, we found this to be true in each brain region examined. In fact, the 20-35% decreases in regional glucose use were just sufficient, in the hyperketonemic rats, to have maintained energy production constant.

Thus, in 20-day-old rats, regional heterogeneity of brain glucose use is similar to that in adult rats. D-β HB use is much less regionally heterogeneous. When brain regional rates of D-β HB use are increased 7-9-fold in acute hyperketonemia, there are compensatory decreases in regional rates of glucose use sufficient to keep regional rates of energy production unchanged.

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314.19 SIMULTANEOUS QUANTITATION OF LOCAL CEREBRAL BLOOD FLOW, GLUCOSE UTILIZATION AND METABOLISM/FLOW COUPLE BY $^{14}$C DOUBLE-LABEL AUTORADIOGRAPHY. M.D. Ginsberg, D.W. Smith*, M.S. Wachtel*, M. Gonzalez-Carvajal*, and R. Busto*. Cerebral Vascular Disease Research Center, Univ. Miami School of Medicine, Miami, FL 33101.

Local glucose metabolism (lCMRgl) and blood flow (lCBF) may be evaluated simultaneously in the animal brain by dual-tracer autoradiography by means of 2-deoxyglucose (DG) and iodoantipyrine (IAP), respectively, provided that activities of the two radiotracers can be resolved on film. The use of beta-emitters for this purpose minimizes problems associated with rapid isotopic decay, radioactive contaminants, limited availability, and increased expense. Using two $^{14}$C-labeled tracers, Furlow et al (JCBF Metab 3:62, 1983) reported complete removal of IAP and complete retention of DG from tissue sections following chloroform wash. However, our preliminary data refuted these findings, showing approx. 6% retention of IAP and 15% disappearance of DG with a 48-hr chloroform wash. This led us to reformulate the method. In preliminary studies, chloroform was found to be superior to toluene, benzene, and 2,2-dimethoxypropane in maximizing retention of DG. In contrast to n-hexane, chloroform permitted retention of equal percentages of DG and DG-6-phosphate, its metabolite. 5-day chloroform wash with continuous agitation reduced IAP by approx. 94% while allowing 60-65% retention of DG. Computer simulations confirmed that propagated error was minimized at high extracted percentages of IAP.

In a series of nitrous oxide-anesthetized rats, lCMRgl and lCBF were determined simultaneously. Single-label IAP- and DG-containing sections were used to quantitate per cent retention of IAP and DG following chloroform wash, and a linear model was used to compute DG and IAP activities, which were converted to lCMRgl and lCBF in the usual manner (Sokoloff et al, J Neurochem 28: 897, 1977; Sakurada et al, Am J Physiol 234: H59, 1978). Pre- and post-wash films were mechanically aligned, scanned by a digitizing densitometer, and the data used to construct pseudo-color displays of lCMRgl, lCBF and the lCMR/lCBF ratio (the metabolism/blood flow "couple"). Region-of-interest measurements were taken simultaneously from all three images via a computer-interfaced image-processor. Values obtained by the double-label strategy were highly correlated with values obtained from comparable single-label animals (r=0.94 for lCMRgl and 0.90 for lCBF). Absolute agreement between double- and single-label results was found to depend critically upon the values assigned to the $^{14}$C-methacrylate standards used for conversion of film optical density to radioactivity units.

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314.20 CORRELATION OF BRAIN FUNCTION AND NEUROANATOMY WITH A COMPUTER STEREOTACTIC ATLAS. L.S. Hibbard*, J.S. McGlone* and R.A. Hawkins (SPON: R.A.W. Lehman). Department of Anesthesia, M.S. Hershey Medical Center, The Pennsylvania State University College of Medicine, Hershey, PA 17033

Neurometabolic experiments using quantitative autoradiography can produce data in amounts large enough to require computer methods for its storage and analysis. Earlier, we reported a 3D reconstruction program system to perform these tasks on rat brain metabolic data and showed that the reconstructed 3D maps of several experiments can be combined to form average maps which may be compared by subtracting one from another, pixel-by-pixel, testing each difference for probable significance (L.S. Hibbard and R.A. Hawkins, Am. J. Physiol. 247:E412, 1984). There remained, however, a need to correlate the reconstructed functional data with neuroanatomy. With our experience combining image data, it appeared that a computerized stereotactic atlas could provide a practical solution to this problem.

Our strategy is to represent neural structures as serial coronal templates, or masks, to be superposed onto the coronal experimental images. The structural masks were created by interactive graphics from a normal control 3D glucose utilization map constructed as the mean of twelve experiments.

To superpose the atlas and experimental data, we define an atlas coordinate system whose origin is located on, and whose z-axis is coincident with, the line defined by the image centers of mass, and whose xy-plane is parallel to the coronal image planes. The z-coordinates of the atlas masks are fixed by the location of the origin and the inter-section spacing of the serial images from which they were taken. Because the experimental data are also reconstructed about the z-axis, superposition requires assigning z-coordinates to the experimental images. Axial sections of the atlas and experimental data in the xz-plane are generated, overlayed, and one is translated with respect to the other by steps along z. The value of the cross-correlation is calculated at each step and the maximum value corresponds to the relative z translation needed to superpose the experimental data onto the atlas. When this operation interleaves the experiment images with the atlas masks, the mask boundary must be interpolated at the experiment image z-coordinate. Once done, the mask is used to sum up the pixel values of the metabolic function over that image. This process, repeated for all experiment images within the z-range of the neural structure of interest, produces the value of the metabolic function within that structure.

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## CYCLIC NUCLEOTIDES II

315.1 IN-VITRO AND IN-VIVO PROTEIN PHOSPHORYLATION IN DROSOPHILA HEADS. J. Buxbaum* and Y. Dudai (SPON: H. Soreq). Department of Neurobiology, The Weizmann Institute of Science, Rehovot 76100, Israel.

Identification of endogenous substrates for cAMP-dependent protein kinase in Drosophila is of interest since mutants exist in this organism that are lesioned in components of the cAMP cascade and also in plasticity (e.g., reviewed in TINS 8(1) 18 (1985)). Such mutations are expected to exert their effects by altering phosphorylation of specific proteins; the identification of the latter may thus shed light on mechanisms of neuronal plasticity. We have analyzed phosphorylation patterns in wild-type Drosophila head preparations, which are a rich source of neural tissue. Two main types of subcellular fractions were used: 1000g sup (S1) and a washed 30000g pellet (P2). Phosphorylation in vitro was assayed by measuring the incorporation of $^{32}$P from [$^{32}$P]γATP into proteins resolved by SDS-PAGE. Ca. 40 phosphoproteins were thus detected in S1, and some of them (MW 16K, 23K, 37K, 50K, 87K, 97K and 165K daltons) displayed cAMP-dependent phosphorylation. In most of our in vitro studies we have concentrated on the P2 fraction. Optimal phosphorylation of this fraction was observed following a 20 sec reaction in the presence of 10mM $Mg^{2+}$ and 10μM ATP. No phosphorylation was detected in the absence of $Mg^{2+}$. cAMP increased the phosphorylation of ca. 10 bands, the most prominent having MW of 17K, 19K, 21K, 36K, 50K, 67K and 92K. The concentration of cAMP producing half-maximal phosphorylation was about 0.3μM, but heterogeneity was detected in the cAMP response; thus the 50K protein started to display a decrease in phosphorylation at cAMP>0.1μM, a behavior characteristic of the regulatory subunit of cAMP-dependent protein kinase. p-hydroxymercuribenzoate (1mM) abolished phosphorylation in the presence of cAMP, whereas in the absence of cAMP only the 50K protein was phosphorylated. This suggests that the kinase requires an active SH and that the regulatory subunit protects the SH, as previously reported for other systems. $F^-$ and vanadate greatly potentiated phosphorylation. $Zn^{2+}$ (5mM), in the absence of $Mg^{2+}$, stimulated cAMP-independent phosphorylation of a different set of proteins. In vivo phosphorylation was studied by feeding flies with carrier-free $^{32}$Pi in sucrose. Optimal incorporation of $^{32}$P into proteins (determined again by SDS-PAGE) was found to occur after 24 hr of feeding. Following feeding, the flies were frozen in liquid $N_2$ and subjected to acetone substitution for 24 hr. Subsequently S1 was prepared. Some 50 phosphoprotein bands were observed, with major multiple bands occuring at ca. 20K, 30K, 50K and 200K daltons. In general, the pattern of phosphorylation in vivo was different from the pattern observed in vitro. Existing mutants in the cAMP cascade are being analyzed using the same in vitro and in vivo techniques. (Supported by the Fund for Basic Research, The Israel Academy of Science, Jerusalem).

315.2 MULTIPLE FORMS OF MEMBRANE-ASSOCIATED PHOSPHOPROTEIN PHOSPHATASES IN DROSOPHILA MELANOGASTER HEADS. Sara Orgad* and Yadin Dudai (SPON: I. Ginzburg). Department of Neurobiology, The Weizmann Institute of Science, Rehovot 76100, Israel.

The phosphorylation state of membrane proteins is assumed to affect various neuronal properties, e.g. ion permeability. Membrane-bound phosphoprotein phosphatases (PPPases) are therefore expected to play a role in the regulation of neuronal activity. Drosophila offers some advantage in studying the structure, regulation and function of PPPases, since mutations might be isolated that should affect specific subpopulations of these enzymes (as is, for example, the case for certain enzymes of the cAMP cascade; reviewed in Trends in Neurosci. 8(1) 18 (1985)). We have set out to characterize membrane bound PPPases in Drosophila as a first step toward the isolation of mutants in these regulatory enzymes. For our studies we are using a washed particulate preparation from fly heads, which is enriched in membranes of neuronal origin. The particulate preparation was treated with 0.2% Triton X-100 to solubilize the enzymes. When aliquots of the detergent-treated preparation were subjected to sucrose gradient centrifugation and the fractions assayed for PPPase activity by using [$^{32}$P]protamine as a substrate, four major peaks were observed, with sedimentation coefficients of ca. 4S, 9S, 10S and 11S, respectively. The 9S peak was usually the predominant one. When p-nitrophenylphosphate was used as a substrate to assay for acid (pH 5.5) or alkaline (pH 8.8) phosphatase, a single major peak of ca. 6S was observed. Triton-treated membrane preparations from the heads of a previously existing putative acid-phosphatase-null mutant and from the heads of a previously existing putative alkaline-phosphatase-null mutant of Drosophila did not reveal a reduction in the level of PPPases activity, indicating that the PPPases detected by us do not share catalytic subunits with acid phosphatase or with alkaline phosphatase (as was suggested for some mammalian systems). The total PPPase activity in Drosophila head membranes was stimulated ca. 2-fold by mM concentrations of $Mn^{2+}$ and of EDTA, was inhibited (by ca. 97%) by mM concentrations of $Zn^{2+}$ and was partially inhibited (by 25-60%) by μM concentrations of $Ca^{2+}$ and vanadate and by mM concentrations of $F^-$. The organophosphate diisopropylfluorophosphate inhibited all the major solubilized PPPase peaks, suggesting that the enzymes contain serine in their active sites. We are currently using antibodies, which have been raised against purified catalytic subunits of PPPase from mammalian brain (i.e., the ca. 35K subunits), for further purification of PPPases from Drosophila head membranes. (Supported by grants from the US-Israel Binational Science Foundation, Jerusalem, and from The Ministry of Health, Jerusalem).

315.3 A CYCLIC AMP-REGULATED MEMBRANE PHOSPHOPROTEIN OF $M_r$ 260,000 LOCALIZED TO CEREBELLAR PURKINJE CELLS. S. I. Walaas, A. C. Nairn*, and P. Greengard. Lab. of Molecular and Cellular Neuroscience, The Rockefeller University, New York, NY 10021.

The second messengers cyclic AMP and cyclic GMP, both of which achieve most or all of their effects through regulation of protein phosphorylation systems, appear to be important in the regulation of neuronal function in the cerebellum (Bloom, F.E., Rev. Physiol. Biochem. Pharmacol., 74:1-103, 1975; Nairn, A.C. and Greengard, P., Fed. Proc., 42:3107-3113, 1983). However, little is known about the exact molecular mechanisms through which cyclic nucleotide-mediated regulation takes place in the cerebellum. In this study, we report the presence of a 260 kilodalton (kDa) neuronal membrane phosphoprotein (PCPP-260, Purkinje Cell Phosphoprotein of 260 kDa) which is a substrate for cAMP-dependent protein kinase and which is highly enriched in cerebellar Purkinje cells.

PCPP-260 was discovered when particulate preparations from rat cerebellum were phosphorylated with activated endogenous or exogenous cyclic AMP-dependent protein kinase. Activation of cyclic GMP-dependent, calcium-calmodulin-dependent or calcium phospholipid-dependent protein kinases did not phosphorylate the protein. We have therefore used cyclic AMP-stimulated phosphorylation as an assay, and found PCPP-260 to be present in all mammalian cerebella so far tested, including human cerebellum. The protein, which can be visualized by Coomassie blue-staining of particulate preparations separated by one- or two- dimensional gel electrophoresis, has been found by peptide mapping studies to have one major phosphorylation site. Analysis of PCPP-260 in cerebella from mutant mice, deficient in either Purkinje cells (pcd, nervous) or granule cells (weaver), indicates that the protein in the cerebellum is restricted to Purkinje cells. Subcellular fractionation of rat cerebella indicates that PCPP-260 is an integral membrane protein. Similar studies of phosphoproteins in other brain regions show that a closely related membrane phosphoprotein of 265 kDa also exists, albeit in much lower concentration, outside the cerebellum. These phosphoproteins may be related to the previouosly described cerebellum-specific glycoproteins $P_{400}$ (Mallet, J. et al., Brain Res., 103:291-312, 1976) or GP-A (Croswall, D.E. and Kelly, P.T., J. Neurochem., 42:534-546, 1984). PCPP-260 may be involved in cyclic AMP-regulated functions in Purkinje cell membranes. (Supported by United States Environmental Protection Agency under Assistance Agreement CR-810608-01).

315.4 EVALUATION OF THE RESPONSE TO CANNABIMIMETIC DRUGS IN NEUROBLASTOMA CELLS. W.A. Devane*, J.W. Spain*, C.J. Coscia and A.C. Howlett* (SPON: M.A. Walz). Depts. of Pharmacology and Biochemistry, St. Louis University School of Medicine, St. Louis, MO 63104.

We have previously demonstrated that the cannabimimetic drugs, $\Delta^9$-tetrahydrocannabinol (THC) and desacetyllevonantradol (DALN), inhibit adenylate cyclase in a neuroblastoma cell line (N18TG2) (Howlett and Fleming, Mol. Pharm. 26:532, 1984). Lymphoma, glioma and certain other neuroblastoma cells failed to respond. Thus, the effects of the cannabimimetic drugs are not universally observed in membranes from all cell types. The inhibition occurred in the nanomolar range, and was immediate and reversible. The effects of the cannabimimetic agents were dependent on the concentrations of guanine nucleotides (half-maximal at 800 nM GTP) and divalent cations (< 1 mM $Mg^{2+}$ or $Mn^{2+}$). Pertussis toxin blocked the inhibition of adenylate cyclase by cannabimimetic drugs. These data suggest that the G proteins mediate the cannabimetic effects.

We examined the interaction between the opioid and cannabimimetic drugs because the opioid drugs also inhibit adenylate cyclase via the G proteins in N18TG2 cells. Delta opioid receptors were detected on N18TG2 plasma membranes using D-$ala^2$-D-$leu^5$-enkephalin (DADLE). No mu or kappa receptors were detected. Neither the binding affinity nor capacity of DADLE were altered in the presence of $\Delta^9$-THC or DALN. Both inhibition of adenylate cyclase in membrane preparations and the decrease in cyclic AMP accumulation in intact cells were produced by the cannabimimetic agents and by the opioid agents, morphine, etorphine, and D-$ala^2$-$met^5$-enkephalinamide. Whereas the effects of the opioids were reversed by naltrexone or naloxone, the cannabimimetic responses were unaffected. The response of maximally effective concentrations of both DALN and etorphine were neither antagonistic, additive, nor synergistic. These results suggest that the opioid and cannabimimetic drugs operate via distinct, noninteracting receptors that can be coupled to the same effector. (Supported by DA03690 and MH40005.)

315.5 EVIDENCE FOR THE INHIBITORY SUBUNIT (Ni) OF ADENYLATE CYCLASE IN NERVOUS AND HEART TISSUE OF APLYSIA. S.D. Critz, J.F. Harper* and J.H. Byrne. Univ. of Texas Medical Sch., Houston, Tx. 77225.

It is now well established that cAMP plays a critical role in modulating a variety of neuronal responses in Aplysia californica. Previous studies have focused on the neurotransmitter activation of adenylate cyclase which presumably occurs via the excitatory GTP-regulated subunit (Ns). An additional site of regulation that has not been examined however, is neurotransmitter activation of the inhibitory subunit (Ni). As a first step in examining this question we show by two techniques that Ni is present in Aplysia tissue. Bordetella pertussis toxin (IAP)-catalyzed ADP ribosylation was used as a specific probe for Ni (Katada and Ui, PNAS 79: 3129, 1982). Desheathed ganglia were homogenized in 1 ml ice cold 50mM Na/Hepes, 1mM EDTA, 0.1 mM ß-mercaptoethanol, 300 mM sucrose, pH 7.6. Two mls of this solution less sucrose were added and the suspension centrifuged at 10,000 RPM for 10 min at 4°C. The pellet was washed in sucrose-free buffer then resuspended in 100 ul IAP-activating buffer (200 mM Tris/Cl, pH 8.0, 2.0mM EDTA, 4 mM $MgCl_2$, 1mM ATP, 2 mM DTT, 0.2mM GTP, 25 ug pyruvate kinase, 15mM $K^+$PEP, and approximately 5 uCi $^{32}$PNAD) with or without 500 ng IAP. This mixture was incubated at 30°C for 60 min. The reaction was then terminated by addition of 100 ul 20% TCA and the samples were gently washed 5 times to remove excess $^{32}$PNAD. Samples were then solubilized, run on 10% SDS gels and prepared for autoradiography by routine methods. We observed a single labeled protein at 40K MW in IAP treated samples. This labeled protein was present in all ganglia tested: abdominal, pleural-pedal, and cerebral. Labeling was also observed in identically prepared heart tissue. In contrast, no label was seen in Aplysia tail muscle. The labeled protein occurs at the same molecular weight as that reported previously for Ni in a variety of other tissues (Manning and Gilman, JBC 258:7059, 1983).

An assay for Ni activity was also performed on neural tissue by measuring adenylate cyclase activity at various GTP concentrations. In other systems in which both Ns and Ni exist a biphasic dose-response relationship to GTP stimulated activity has been found (Rodbell, Nature 284:17, 1980). We observed a stimulatory effect of GTP on adenylate cyclase at low concentrations of GTP, which peaked at approximately 100 uM. With higher concentrations of GTP inhibition of activity was observed, suggesting that Ni inhibitory activity overcomes Ns stimulation of adenylate cyclase.

These findings provide preliminary evidence that Ni is present in Aplysia tissue. Ni may serve as an important site for the regulation of cAMP synthesis and perhaps neuronal plasticity.

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315.6 ASSOCIATION OF KINASES AND OTHER PROTEINS IN THE cAMP-CASCADE WITH THE MEMBRANE-CYTOSKELETON OF APLYSIA NEURONS. T. Saitoh, M. Schonberg*, J.H. Schwartz, Howard Hughes Med. Inst., Columbia Univ. Coll. Phys. & Surgeons, New York, NY 10032.

Extracting with 0.1% NP-40 in 2M glycerol, Solomon and his colleagues (Cell 18:431, 1979) obtained a cytoskeleton fraction lacking membrane but containing loosely associated proteins. We adapted this method by using 0.1% saponin, which does not solubilize membrane, to prepare a membrane-cytoskeleton complex in order to study the compartmentalization of $Ca^{++}$/calmodulin-dependent kinase II (PNAS 80:6708, 1983). Conditions that lead to cAMP-dependent or $Ca^{++}$/calmodulin-dependent phosphorylation were found to dissociate this kinase from the complex (J. Cell Biol. 100:835, 1985). We now have characterized both the membrane-cytoskeleton complex (residue fraction) and the extract produced by this procedure.

The membrane-cytoskeleton complex contained all of the proteins ($M_r$ 90,000, 55,000, 40,000, 35,000, and 24,000) that bind $^{125}$I-$Ca^{++}$/calmodulin kinase as determined by Western blotting. Half of the total regulatory subunits of the cAMP-dependent kinase were also associated with the complex. This association is selective, because a characteristic subset of the five neuronal 8-$N_3$-cAMP-binding species was preferentially attached. The complex contained essentially all of the newly-synthesized glycoproteins labeled after introsomatic injection of $^3$H-fucose into the giant cerebral neuron, indicating that the internal membrane systems of the cell (Golgi and smooth endoplasmic reticulum) is preserved. Indeed, the complex appeared quite similar to untreated cells when examined by electron microscopy, even though 37% of the total protein was extracted. Plasma membrane also was preserved: the complex was enriched in membrane glycoproteins that bind concanavalin A. Although more than half of the adenylate cyclase activity is not recovered after the extraction, we found 60% of the remaining enzyme in the complex together with putative Gs ($M_r$ 55,000) and Gi ($M_r$ 41,000) subunits. These were $^{32}$P-ADP-ribosylated in the presence of cholera toxin or pertussis toxin.

Some particulate material is extracted from nerve cells by saponin. A fraction sedimented from the saponin extract at 100,000 xg for 1 h contained neurofilaments, rough endoplasmic reticulum, and small bits of membrane, but no $^3$H-fucosyl glycoproteins. The parts of the cell from which this fraction is derived are uncertain, but it contained about 10% of the total concanavalin A binding activity and 40% of the remaining cyclase, and these appear to have distinctive properties.

315.7 TRANSDUCING PROTEINS AND THE REGULATION OF ADENYLATE CYCLASE IN APLYSIA NEURONS. M. Schonberg*, A. Stapleton*, T. Saitoh, Howard Hughes Medical Institute, Columbia Univ. Coll. Physicians & Surgeons, New York, NY 10032.

Application of serotonin increases the synthesis of cAMP in Aplysia neurons and stimulates adenylate cyclase in membranes isolated from nervous tissue. Because it is dependent on GTP, stimulation of cyclase by serotonin probably is mediated by a stimulatory G transducing protein (Gs). A further indication for an Aplysia Gs subunit is that cholera toxin stimulates cyclase activity in membranes isolated from Aplysia ganglia and causes the ADP-ribosylation of an $M_r$ 55,000 protein that is membrane-associated. We now report evidence for an inhibitory G subunit of cyclase (Gi).

Pertussis toxin produces the $(^{32}P)$ADP-ribosylation of an $M_r$ 41,000 protein in Aplysia neuronal membranes. It is thought that ADP-ribosylation inactivates Gi in other animals. We find that cyclase activity is markedly inhibited at concentrations of GTP-gamma-S greater than $10^{-3}$M; this inhibition is blocked by pertussis toxin, as would be expected if the Aplysia Gi is inactivated. In adddition, pertussis toxin potentiates the activation of the cyclase produced by suboptimal concentrations of serotonin.

We partitioned neuronal membranes into two fractions (accompanying abstract). The proportion of adenylate cyclases of putative Gs to Gi, revealed by the $(^{32}P)$ADP-ribosylation produced by cholera toxin or by pertussis toxin, was quite different in these two fractions. The properties in the two particulate fractions seem to differ from each other. The stimulation of cyclase activity by various effectors (GTP-gamma-S, $MnCl_2$, cholera toxin, serotonin, and $Ca^{++}$/calmodulin) in the two different membrane fractions was different. The degree of the interference of $Mn^{++}$ stimulation, GTP-gamma-S stimulation of cyclase activity by pertusis toxin and cholera toxin was different in the two fractions. The distinctive behavior of the cyclase in different membranes might partly be explained by differences in the proportion of the two types of transducing subunits.

315.8 PHOSPHODIESTERASE AND ADENYLATE CYCLASE ACTIVITY IN APLYSIA BAG CELL NEURONS: EFFECT OF CALCIUM AND A CALMODULIN ANTAGONIST. C.L. Bruehl & R.W. Berry. Dept. Cell Bio. & Anat. Northwestern Univ Medical School, Chicago, IL 60611

In the bag cell neurons of Aplysia, cyclic AMP and calcium are known to be crucial for normal electrical and biochemical function (Kaczmarek & Strumwasser, J.Neurophysiol.,52:340, 1984; Bruehl & Berry, J.Neurosci., in press). Since, in other systems, calcium regulates cAMP levels, we looked for such an interaction by assaying the $Ca^{2+}$ sensitivity of phosphodiesterase (PDE) and adenylate cyclase in homogenates of bag cells. In a 0 $Ca^{2+}$/EGTA medium, PDE activity was measured at $3.4 \pm 0.6 \times 10^{-8}$ moles cAMP hydrolyzed/min/mg protein. When EGTA was replaced in the medium by varying amounts of $CaCl_2$, PDE activity was steadily increased (see below), rising to almost 3 times controls at 300 uM. When the calmodulin inhibitor R 24 571 was included in the reaction along with calcium, PDE activity was markedly reduced. R 24 571 had little effect on PDE activity when assayed in a 0 $Ca^{2+}$/EGTA medium. While stimulation is seen only at relatively high calcium concentrations, the results suggest the presence of at least one calcium/calmodulin-sensitive form of phosphodiesterase in the bag cells. In addition, $Ca^{2+}$ stimulated adenylate cyclase activity. If these effects accurately reflect the situation in vivo, then calcium entry during bag cell discharge would be expected to increase cAMP turnover. Supported by NSF BNS-82- 19630.

Calcium Sensitivity of Bag Cell PDE and Adenylate Cyclase

| $[Ca^{2+}]$ | PDE (moles cAMP hydrolyzed/min/mg) | Adenylate Cyclase (moles cAMP/min/mg) |
|---|---|---|
| 0 $Ca^{2+}$/EGTA | $3.4 \pm 0.6 \times 10^{-8}$ | $10.2 \pm 0.7 \times 10^{-12}$ |
| + R 24 571 | $3.7 \pm 0.2 \times 10^{-8}$ | ---------- |
| 1 uM | $3.7 \pm 0.2 \times 10^{-8}$ | $10.9 \pm 0.1 \times 10^{-12}$ |
| 10 uM | $4.5 \pm 0.3 \times 10^{-8}$ | $14.9 \pm 0.4 \times 10^{-12}$ |
| 30 uM | $6.5 \pm 0.2 \times 10^{-8}$ | $16.1 \pm 0.5 \times 10^{-12}$ |
| + R 24 571 | $3.1 \pm 0.1 \times 10^{-8}$ | ---------- |
| 100 uM | $8.5 \pm 0.4 \times 10^{-8}$ | $17.0 \pm 0.3 \times 10^{-12}$ |
| 300 uM | $1.1 \pm 0.5 \times 10^{-7}$ | $39.2 \pm 1.8 \times 10^{-12}$ |
| + R 24 571 | $6.9 \pm 0.6 \times 10^{-8}$ | ---------- |

315.9 CALMODULIN ACTIVATES ADENYLATE CYCLASE FROM RAT ADENOHYPOPHYSIS. D.V. Greenlee. Dept. of Zoological and Biomedical Sciences and the College of Osteopathic Medicine, Ohio University, Athens, OH 45701.

Calmodulin (CaM) is a ubiquitous, $Ca^{2+}$ binding protein which activates numerous types of enzymes upon formation of the $(Ca^{2+})_n$•CaM•enzyme complex. A few adenylate cyclases, for example, from bovine brain and from Bordetella pertussis (Greenlee, et al. (1982) Biochemistry 21:2759), are known to be calmodulin responsive. Recent literature suggests that adenylate cyclase from rat anterior pituitary is also CaM sensitive, although a detailed analysis of the calmodulin specificity for the enzyme has not been reported. The current results corroborate the existence of a CaM-sensitive adenohypophyseal cyclase and characterize in detail the $Ca^{2+}$ sensitivity of the enzyme.

Adenohypophyseal membranes were prepared by homogenization of adult male Sprague-Dawley rat pituitaries in buffer containing 1mM EGTA. Following centrifugation of the membranes, the pellet was washed once more with EGTA and resuspended for assay of adenylate cyclase activity. This treatment apparently removed endogenous CaM and further washing did not enhance cyclase stimulation by added calmodulin. In the presence of 200μM EGTA, addition of $CaCl_2$ caused only a dose-dependent inhibition of basal cyclase activity. In the presence of 200μM EGTA and CaM, $CaCl_2$ addition caused a biphasic response of cyclase activity—low $Ca^{2+}$ concentrations stimulated cyclase while higher concentrations inhibited the enzyme. In contrast to brain cyclase, which is activated by nanomolar concentrations of CaM, adenylate cyclase from anterior pituitary required micromolar CaM for activation. Consequently, $Ca^{2+}$ dose-response curves for calmodulin activation were shifted to higher concentrations as CaM levels were increased. For example, employing calmodulin concentrations of 10μM, 50μM, and 100μM, optimal added $Ca^{2+}$ concentrations were 125μM, 250μM, and 350μM, respectively. Using optimal $Ca^{2+}$ concentrations for each concentration of CaM, calmodulin activated pituitary cyclase in a dose-dependent manner. Fluphenazine (50μM), a phenothiazine drug known to bind CaM in the presence of $Ca^{2+}$, completely inhibited calmodulin (2μM) activation of adenylate cyclase. Excess EGTA (5mM) also completely abolished CaM activation of cyclase activity. These results indicate that 1) a calmodulin-sensitive adenylate cyclase exists in rat anterior pituitary and 2) this enzyme differs from brain cyclase in its responsiveness to calmodulin.

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315.10 PROTEIN KINASE C ACTION ON HORMONE RECEPTOR ACTIVITY IN PITUITARY CELLS. S.T. Summers*, P.L. Canonico and M.J. Cronin, Dept. of Physiology, Univ. of Virginia, Charlottesville, Virginia 22908.

Tumor promoters (TP) are capable of substituting for diacylglycerols to activate the calcium and phospholipid-dependent, protein kinase C. In determining if these agents affected hormone receptor activity in anterior pituitary (AP) cells, we found a potentiation of growth hormone (GH) releasing factor (GRF) stimulated GH release and an abolition of dopaminergic inhibition of prolactin release (Eur J Pharmacol 111: 1985, in press). To investigate the mechanism for such effects, we then studied GRF receptor coupling to the cyclic AMP (cAMP) generating system. TPs markedly potentiated maximal GRF-initiated cAMP accumulation (2.1 ± 0.2-fold at 15 min). This was specific, in that it was produced by the TPs phorbol myristate acetate (PMA), phorbol dibutyrate (PDB) and the non-phorbol teleocidin, whereas a phorbol that is inactive as a TP (4-alpha-phorboldidecanoate) had no effect. Furthermore, a synthetic diacylglycerol, 1-oleoyl-2-acetyl glycerol mimicked the action of TPs. This suggested that protein kinase C activity could modulate the coupling of GRF receptor to the adenylate cyclase system in these heterogenous AP cells. In order to more precisely define this mechanism, we turned to the 235-1 cell line derived from a prolactin-secreting rat AP tumor. These cells respond to prostaglandin E1 (PGE1) and forskolin with an increase in cellular cAMP and prolactin release. The cells also show enhanced prolactin secretion after exposure to TPs. Our original observations in normal AP cells of potentiated cAMP responses to secretagogues after TPs has been confirmed in 235-1 cells. Cotreatment with PMA or PDB potentiated the cellular accumulation of cAMP in response to PGE1 by 78% at 10 minutes. TPs also increased cAMP levels in response to forskolin by 36% at 10 minutes. While TPs did not directly affect adenylate cyclase activity in membrane preparations, membranes from cells pretreated with PMA or PDB have increased basal and forskolin-stimulated adenylate cyclase activity.

| | Adenylate Cyclase Activity (pmoles/mg/min) | |
|---|---|---|
| Forskolin (uM) | Vehicle Pretreat | PMA (100 nM) Pretreat |
| 0 | 113 ± 1 | 166 ± 9 |
| 0.1 | 247 ± 17 | 316 ± 4 |
| 1.0 | 499 ± 19 | 734 ± 17 |
| 10.0 | 1090 ± 40 | 1700 ± 40 |
| 100.0 | 2690 ± 70 | 4100 ± 13 |

In conclusion, we have demonstrated that TPs, presumably via the activation of protein kinase C, can potentiate cAMP and secretory responses to hormonal stimulation in AP cells. The ability of TPs to amplify the response to hormones as well as forskolin suggests a site of TP action at the regulatory and/or catalytic subunits of the adenylate cyclase holoenzyme. (RCDA1K04NS00601, AM32632, NS18409, AM22125, ACS149B)

315.11 cAMP-DEPENDENT PROTEIN KINASE ACTIVITY AND PROTEIN PHOSPHORYLATION IN AtT-20 CELLS. G. Rougon*, J. Barbet*, H.U. Affolter* and T. Reisine. (SPON: J. Axelrod). Lab. of Cell Biology, National Institute of Mental Health and Lab. of Mathematical Biology, National Cancer Institute, Bethesda MD 20205.

The AtT-20/D16-16 tumor cell line is derived from the mouse anterior pituitary and is capable of synthesizing and secreting adrenocorticotropin (ACTH). Both of these functions are stimulated by a variety of physiological and pharmacological agents. In this study, we investigated the possible role of cAMP-dependent protein kinase as well as other kinases in the regulation of ACTH formation and release. AtT-20 cells were preincubated with $^{32}PO_4^{3-}$ for 3 to 4 hr at 37°C and then stimulated for 10 min by adding to the radioactive medium forskolin (an activator of adenylate cyclase) or phorbol ester (a stimulator of protein kinase C). After lysis of the cells and subcellular fractionation, the cytoplasmic, nuclear and membrane fractions were analyzed by single and two-dimensional gel electrophoresis. Forskolin increased the phosphorylation of several proteins among which the most affected were three at 70-75 Kdaltons (pI≈ 4.5, 6 and 7), one at 40 Kdaltons (pI≈ 5) and two at 34 Kdaltons (pI≈ 4.5 and 6.5) in the cytoplasm, one at 44 Kdaltons (pI≈ 8) and one at 28 Kdaltons (pI≈ 7.5) in the membrane, and one at 75 Kdaltons (pI≈ 7.5) and one at 22 Kdaltons (pI≈ 9) in the nucleus. The protein phosphorylation induced by forskolin was dramatically reduced by the heat-resistant inhibitor of cAMP-dependent protein kinase (Walsh's inhibitor) delivered intracellularly by targeted liposomes. Like forskolin, phorbol esters also increased the phosphorylation of many proteins but appeared to specifically induce the phosphorylation of a protein of ≈ 20 Kdaltons (pI 6-7) in the cytoplasmic fraction. Thus, both cAMP-dependent protein kinase and protein kinase C regulate the phosphorylation of different as well as similar cellular proteins. The role of these phosphorylation events in the control of ACTH synthesis and release by physiological stimuli is presently under study.

315.12 EFFECT OF ANGIOTENSIN II ON ADENYLATE CYCLASE ACTIVITY IN NEUROBLASTOMA X GLIOMA HYBRID CELLS. J.E. Watkins* and J.A. Weyhenmeyer (SPON: C. Hockman). College of Medicine, University of Illinois, Urbana, IL 61801.

Physiological and pharmacological studies have suggested that angiotensin II (ANG II) plays a role in the neuromodulation of blood pressure, fluid intake, hormone release and memory. Although the existence of specific high affinity ANG II binding sites in brain has been well documented, there is a general paucity of information regarding the cellular (physiological) effect of ANG II ligand/receptor interaction. Gilbert et al. (Biochem. Pharm. 33: 2527, 1984) have reported that angiotensin I, II, and III stimulate a calcium-dependent increase of cGMP in murine neuroblastoma cells (N1E-115). We have recently demonstrated that a neuroblastoma x glioma hybrid cell line, NG108-15, contains high affinity ANG II binding sites that have similar kinetic characteristics to ANG II receptors in mammalian brain (Brain Res. Bull., in press, 1985). The purpose of this study was to examine the effect of ANG II/ANG II receptor interaction on adenylate cyclase activity in the NG108-15 hybrid cells.

Neuroblastoma x glioma hybrid cells were grown to confluency in Falcon tissue culture plates containing 50 ml of Dulbecco's Modified Eagles medium (DMEM) with 10% fetal calf serum, under conditions of 90% air and 10% $CO_2$ at 37° C in a humidified atmosphere. The cells were challenged with 25 mM ANG II in DMEM containing 100 mM 3-isobutyl-5-methylxanthine (IBMX) or DMEM/IBMX alone (control). Triplicates of ANG II-stimulated and control samples were taken between 10 and 110 sec at 20 sec intervals, and prepared for analysis according to the method of Fletcher et al. (BBRC 70:1297, 1976). The resulting aliquots of cell lysates were analyzed by high performance liquid chromatography using a Servachrome Si100:polyol:DEAE column with a mobile phase of 200 mM $NaH_2PO_3$. Standard cAMP, ANG II-stimulated and control samples were injected, monitored at 254 nm, and the retention time and plate heights recorded. The quantitative amounts of cAMP were determined by plotting plate height vs injection quantity of the cAMP standard using linear regression analysis. Our results demonstrate a significant time-dependent increase in cAMP levels of ANG II-stimulated cells as compared to controls. At 50 and 110 sec after ANG II-stimulation, cAMP levels were 35% and 300% higher that control values, respectively. These data suggest that ANG II/ANG II receptor interaction stimulates adenylate cyclase activity in brain cells, and provide additional support for the proposed transmitter/modulator role for ANG II in CNS tissues.

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315.13 INTRACELLULAR INJECTION OF cGMP-DEPENDENT PROTEIN KINASE RESULTS IN INCREASED INPUT RESISTANCE IN NEURONS OF THE MAMMALIAN MOTOR CORTEX. T. Bartfai*, C.D. Woody, E. Gruen*, A. Nairn* and P. Greengard (SPON: D. Birt). UCLA Medical Center, Los Angeles, CA 90024, and The Rockefeller University, New York, NY 10021.

Intracellular pressure injection of purified, cGMP-dependent protein kinase into neurons of the precruciate cortex of awake cats caused increases in the input resistances (Rm) of 20 out of 26 injected cells, measured with 1nA, 40ms, rectangular, bridge balanced, hyperpolarizing and depolarizing pulses. The mean input resistance increased within seconds (as rapidly as measurements could be made) after injection from 6.4 ± 1.1 (S.E.M.) Mohms to 11.2 ± 2.1 Mohms and remained elevated for two minutes or longer. In these experiments the cGMP-dependent protein kinase was incubated with 10 micromolar cyclic GMP 30 min prior to filling the electrodes. Pressure injection of the cGMP-dependent protein kinase without preincubation with cGMP in 17 other cells caused smaller increases in Rm that were slower in onset, reaching a maximum value 60-90 seconds after injection. Only one of 26 "control-cells" injected with heat inactivated cGMP-dependent protein kinase, with (n=8) or without (n=18) pre-incubation with 10 micromolar cGMP, showed increased input resistance; all the others showed no change or small decreases in Rm over the 2 min period of observation. Up to sixty-five percent of cells of this same cortical area given extracellular application of acetylcholine or intracellular application of 1 mM cGMP in earlier studies showed increases in input resistance of a magnitude comparable to that observed on injection of the activated, cGMP-dependent protein kinase. The results indicate that intracellular injection of the cGMP-dependent protein kinase into neurons of the precruciate cortex of the awake cat can mimic the actions of extracellularly applied acetylcholine and intracellularly applied cGMP.
(Supported by AFOSR and USPHS grants and a Fullbright fellowship to T.B.)

315.14 ACTIVATION OF ADENYLATE CYCLASE IN CULTURED MOUSE DORSAL ROOT GANGLION NEURONS; INVOLVEMENT OF POTASSIUM CONDUCTANCE: D.S.Grega & R.L.Macdonald, Dept. of Neurol, Univ. of Mich., Ann Arbor, MI, 48109.

Modulation of neuronal activity in some vertebrate and invertebrate systems is the result of alterations in the cyclic AMP (cAMP)-second messenger system. Adenylate cyclase (AC) is pivotal for its activation. To stimulate this system at various levels, we are using a number of compounds to alter the activity and excitability of cultured mouse dorsal root ganglion neurons (DRG).

Cell culture and intracellular recording techniques are as previously described (Werz & Macdonald, J.PET 227:394, 1983). Some neurons are impaled or reimpaled (after potassium acetate impalement) with a micropipette filled with cesium acetate (CsAc) and cesium, a potassium channel blocker, is injected intracellularly. Drugs are applied to single neurons by pressure ejection from micropipettes with tip diameters of 2-5 μm. Stock drug solutions are made in saline except for forskolin (FOR) and prostaglandin $E_1$ ($PGE_1$) which are dissolved in DMSO. DMSO control in saline does not significantly alter action potential duration (APD).

Forskolin (FOR), an adenylate cyclase (AC) activator, initially prolongs action potential duration (APD) with a decrease in after hyperpolarization (AHP) and subsequently shortens (APD) with an increased AHP with 1-2 sec puffs in a concentration dependent fashion from 100μM with 361% prolongation with 55.5% shortening to 1 μM with 16.2% prolongation with 4.7% shortening. FOR does not produce a significant change in resting membrane potential (-60mV) conductance. Cholera toxin (CT, 10 μg/ml), which activates AC by binding to the $G_s$ protein and $PGE_1$ (1 μM) which activates AC via the membrane surface receptor, mimic the FOR effect of prolongation and shortening. APD prolongation persists with longer (sec to min) application of FOR or CT. Analogs of cAMP, dibutyryl cAMP (100 μM) and 8-Bromo-cAMP (1 mM), which mimic the end product of increased AC activity, produce APD prolongation and shortening. 2', 5'-dideoxyadenosine (DDA), an AC inhibitor, partially inhibited the FOR-induced APD prolongation (38%) with mixed results on the subsequent shortening. Intracellular injection of Cs blocked the FOR induced APD prolongation (96%) with predominantly a reduction in the subsequent APD shortening (91%) indicating the involvement of a potassium conductance.

The data suggest that the APD prolongation is due to an immediate increase in AC and cAMP which eliminates a voltage or calcium-dependent potassium conductance. The APD shortening appears to be either a reaction to the prolongation or a more temporally distant result of increased AC/cAMP. Single electrode voltage clamp and patch clamp techniques will be employed to further elucidate this biphasic phenomenon.
Supported by NIH fellowship F32 NS07231.

315.15 UPTAKE-DEPENDENT SEROTONERGIC STIMULATION OF cGMP LEVELS IN HUMAN PLATELETS DURING ELECTROCONVULSIVE TREATMENT. M.-L. Tjornhammar*, B. Martenson*, M. Asberg* and T. Bartfai* (SPON: B. Howard). Department of Biochemistry, Arrheniuslaboratory, 10609 Stockholm and Department of Psychiatry, Karolinska Hospital, 10401 Stockholm.

Elevation of cGMP levels in human platelets by serotonin has earlier been reported by several laboratories but the molecular mechanism of this response was not known. Unlike other neurotransmitter mediated increases in cGMP levels, this process was not dependent on the presence of extracellular Ca-ions. It did not involve the type 1 and 2 serotonin receptors on platelets, but it could be blocked by the serotonin uptake inhibitors, imipramine and alaproclate. Concentration dependent stimulation of cGMP levels by serotonin was only observed in the presence of $Na^+$ and $Cl^-$ ions and at temperatures above 18°C, indicating the requirement for an active 5-HT carrier. Thus, measurements of the 7-10 fold serotonin mediated increases in cGMP levels may serve as sensitive indicators of the changes in the serotonin uptake system that has intensively been studied in depression.

The high sensitivity of the method permits use of the small volume blood samples that were drawn before, during and after consecutive sessions of electroconvulsive treatment given to depressed patients. (Supported by the Swedish Medical Research Council and NIMH grants.)

315.16 EFFECTS OF MODIFICATIONS OF THE 4-(3,4-DIHYDROXYPHENYL)-1,2,3,4-TETRAHYDROISOQUINOLINE STRUCTURE ON DOPAMINE SENSITIVE RAT RETINAL ADENYLATE CYCLASE ACTIVITY. L. L. Truex, M. M. Foreman, R. M. Riggs*, and D. E. Nichols. The Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285 and Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, IN 47907.

The effects of dopamine on various physiological and biochemical processes are attributed to the stimulation of either a $D_1$ or $D_2$ receptor. One biochemical index of the $D_1$ receptor response is the amplification of dopamine-sensitive adenylate cyclase activity. The purpose of these studies was to examine the effects of modifications of the 4-(3,4-dihydroxy-phenyl)-1,2,3,4-tetrahydroisoquinoline structure on rat retinal adenylate cyclase activity. Rat retinas were homogenized in 150 vol/wt of 2.0 mM Tris · HCl pH 7.4 with 2 mM EGTA using a teflon-glass homogenizer. Each reaction mixture contained the following final concentrations in a volume of 0.2 ml:2mM $MgSO_4 \cdot 7\ H_2O$, 0.5 mM EGTA, 1 mM IBMX, 0.01 mM GTP, 80 mM Tris · HCl (pH 7.4), 0.5 mM ATP with approximately 5 x $10^6$ DPM of $^{32}$P-ATP and 20-30 µg retinal homogenate protein. Following an incubation of 20 min at 30°C, the reaction was terminated by adding 200 µl of a solution containing 1% SDS, 20 mM ATP, 0.7 mM cyclic AMP with 1.0 x $10^4$ DPM $^3$H-cyclic AMP in 80 mM Tris · HCl pH 7.4 and heating to 85°C for 2 min. Cyclic AMP was isolated from the mixture using the column chromatographic technique of Salomon (Adv. Cyc. Nuc. Res., 10:35-55, 1979). In these studies, the major structural modifications that were evaluated included alteration of the N-side chain length, substitution of a thiophene for the isoquinoline and removal of one hydroxyl group of the catechol ring. Elongation of the N-side chain length resulted in a progressive decline in $D_1$ agonist activity with H > Me > Et > Pr. The substitution of a thiophene for the isoquinoline resulted in an approximate 10-fold increase in $D_1$ agonist activity, indicating that the type of ring structure associated with the phenethylamine pharmacophore can influence the expression of $D_1$ agonist activity. The modification of the catechol ring to a monohydroxyphenyl analog resulted in greatly diminished $D_1$ agonist activity indicating an absolute requirement of a catechol structure for maximal activity. Finally, the $D_1$ agonist effects of the N-unsubstituted dihydroxyphenyl analogs were confirmed by the concentration dependent attenuation of the adenylate cyclase response by SCH23390, a selective $D_1$ antagonist.

## MOLECULAR BIOLOGY OF GENE EXPRESSION AND NUCLEIC ACIDS IV

316.1 THE DISTRIBUTION OF TYROSINE HYDROXYLASE HOMOLOGOUS RNA IN THE CENTRAL NERVOUS SYSTEM OF THE RAT. T.J.Mahalik, N. Chaudhari, W.E. Hahn and T.E. Finger. Dept. of Anatomy, U. of Colorado Med. School, Denver, CO.

Tyrosine hydroxylase (TH) is the rate limiting enzyme in the catecholamine biosynthetic pathway. In rat PC8b cells, induction of TH enzyme activity by 8-bromo cAMP and dexamethasone is directly related to an increase in levels of TH-homologous mRNA (Lewis et al. J. Biol. Chem. 258:14632:83). Although the TH gene appears to be regulated at the transcriptional level in PC cells, very little is known about the manner in which TH expression is controlled in the central nervous system (CNS). The purpose of our study was to map the distribution of TH-homologous sequences in the rat brain at the regional and single cell level in a series of dot blot and in situ hybridization experiments.

For dot blots, dissected regions of the CNS from adult Sprague-Dawley rats were homogenized and were blotted onto nitrocellulose with sodium iodide (Bresser et al. DNA 2:243:83). Slide-mounted tissue sections were prepared for in situ hybridization by standard methods. RNA transcripts of a TH cDNA, containing both coding and non-coding TH-homologous sequences (Lewis et al. J.Biol. Chem. 258:14632:83), were synthesized in vitro with SP6 polymerase, and were used as probes in hybridization experiments.

We observed TH homologous RNAs in the olfactory bulb and substantia nigra. In addition, we observed positive hybridization signals in the cerebral cortex, the cerebellar cortex, and the caudate nucleus; these areas normally do not contain catecholaminergic cell bodies. In situ hybridization revealed the presence of TH-homologous sequences in the cortex and in the granule cell layer of the hippocampus. Control hybridizations with pBR322 transcripts or with sense transcipts of the TH cDNA resulted in only background labeling in both dot blot and in situ hybridization experiments.

The results of our experiments suggest that TH-homologous RNAs are present in CNS regions which do not contain catecholaminergic cell bodies. Our findings may mean that the TH gene is under loose transcriptional control, or that numerous transcripts in the CNS contain a sequence that is homologous to a portion of TH mRNA. We are currently carrying out experiments to resolve the issues raised by our initial observations.

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316.2 SEQUENCE HOMOLOGY BETWEEN TYROSINE HYDROXYLASE mRNA AND A RAT ADRENAL cDNA. S. Edmonds* and K.L. O'Malley* (Sponsor: K. Isenberg), Department Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

As part of our ongoing project to characterize catecholamine biosynthetic enzymes, we have isolated a cDNA clone from a rat adrenal medulla library by cross hybridization with a rat tyrosine hydroxylase (TH) clone. The TH clone was isolated from the rat pheochromocytoma cell line, PC 12. Nitrocellulose filters of both adrenal and PC 12 size fractionated mRNAs were hybridized with the adrenal clone. A major band of 5.6 kb was detected in both RNA species, as well as a band of 1900 bp. The 1900 bp band co-migrated with TH mRNA as determined by hybridization with a nick translated TH cDNA. Southern blot analysis revealed several hybridizing bands homologous to the adrenal clone. A 9.5 kb rat genomic band hybridizing intensely with the adrenal cDNA also hybridized with the rat TH probe. These results demonstrate that the adrenal clone shares sequences in common with the TH gene and/or is closely linked to it in the genome. Hybrid selected mRNA translation products are immunoprecipitated with both dopamine beta-hydroxylase and phenylethanolamine N-methyltransferase antisera suggesting this clone may code for another catecholamine pathway enzyme.

316.3 TISSUE SPECIFIC EXPRESSION OF TYROSINE HYDROXYLASE RNA. E.J. Lewis, M.L. Delano*, W.R. Aldrich*. Neuroscience Program, Tufts Univ. Med. Sch., Boston, MA 02115

Tyrosine hydroxylase (TH), the rate limiting enzyme for synthesis of catecholamines, has a tissue specific distribution for its synthesis. TH is synthesized in cell bodies present in the adrenal medulla, the sympathetic ganglia and certain nuclei of the brain. To determine whether the pattern of distribution of TH RNA is the same as that of TH-synthesizing cell bodies, RNA was extracted from various tissues in rat and analyzed by Northern blot. RNA was extracted from the midbrain tegmentum and olfactory bulb, both of which contain dopaminergic neurons and from the midbrain tectum, the cerebellum and the frontal lobe, regions that do not contain catecholaminergic cell bodies. The probe used contained 280 bases of $^{32}$P-labelled antisense TH RNA, corresponding to a translated portion of mRNA$^{TH}$. This probe was derived from the cDNA clone pTH.4 (J. Biol. Chem. 258:14632, 1983) which was subcloned into the SP65 vector. Following hybridization of Northern blots, a band of 1800-1900 nucleotides was observed in total RNA extracted from the adrenal gland, the superior cervical ganglia, the midbrain tegmentum and the olfactory bulb. No qualitative differences were observed in the migration of the putative mRNA$^{TH}$ from these tissues. There was no band corresponding to mRNA$^{TH}$ in RNA extracted from frontal lobe, the midbrain tectum or the cerebellum. In all areas of the brain tested, hybridization was also observed to a series of distinct bands of molecular weights higher than that of mRNA$^{TH}$. The migration of these RNAs was apparently identical for RNA from all brain areas and the relative intensity of the bands (per ug RNA) was greatest in the cerebellum. These RNAs were far less abundant in RNA extracted from liver or kidney, although three of these RNAs were prominent in heart RNA. These bands of hybridization persisted despite stringent washes of filters in 7.5 mM NaCl at 63$^{o}$C. When RNAs were probed with an anitsense TH RNA transcript containing only 110 bases of the original 280 base probe, hybridization was confined to the 1800-1900 base mRNA$^{TH}$, observed only in RNA extracted from tissues which contain TH cell bodies. It is thus unlikely that the high molecular weight cross-hybridizing RNA species represent partially processed precursors to mRNA$^{TH}$, because they do not appear when the 110 base probe is used.

The results of these experiments suggest that TH RNA is specifically localized to areas which synthesize TH protein. Although the specific nature of cross-hybridization of the TH probe to other RNAs is not known, it is likely that these bands represent RNAs containing a short region of sequence homology to mRNA$^{TH}$, but may be functionally unrelated. Caution should be taken in interpretation of experimental results when pTH.4 is utilized as a probe for brain RNA that is not separated by size, as in dot blot or in situ hybridization. (Supported by NIH grant GM3991)

316.4 REGULATION OF TYROSINE HYDROXYLASE BY NERVE GROWTH FACTOR AND CELL-CELL CONTACT IN PRIMARY CULTURES OF BOVINE ADRENAL CHROMAFFIN CELLS. S. Saadat*, J. Mallet and H. Thoenen (SPON: H. Thoenen) Dept. of Neurochemistry, Max-Planck-Institute for Psychiatry, D-8033 Martinsried, FRG.

We have previously reported that the specific activity of tyrosine hydroxylase (TH) was increased by nerve growth factor (NGF) (Acheson, A.L., Naujoks, K. and Thoenen, H. (1984) J. Neurosci. 4, 1771-1780) and cell-cell contact (Acheson, A.L. and Thoenen, H. (1983) J. Cell Biol. 97, 925-928) in bovine adrenal chromaffin cultures. The increase of TH by cell contact was inhibited by α-amanitin, whereas the increase of TH by NGF was not inhibited. Cycloheximide inhibited the increase of TH in both cases. This suggested that TH synthesis was induced at the transcriptional level by cell contact and at a posttranscriptional level by NGF.

To study the induction in more detail at the mRNA level, a cDNA probe specific for bovine TH was prepared. Total RNA was isolated from chromaffin cultures, separated by agarose gel electrophoresis and the RNA was blotted onto nitrocellulose. Hybridization with nick-translated TH cDNA revealed a band with a size of 1.8 kb. Quantitative changes in the TH mRNA levels in chromaffin cultures are investigated after the addition of NGF, or by increasing the cell density of chromaffin cells to 300,000 cells/cm$^2$ by replating cells that were kept at a density of 30,000 cells/cm$^2$.

316.5 shi$^{mld}$*jp$^{msd}$ and shi*jp DOUBLE MUTANT MICE : UNEXPECTED INTERGENIC INTERACTIONS OF ALLELIC HYPOMYELINATION MUTATIONS. S. Billings-Gagliardi, M.E. Pomeroy*, and M.K. Wolf. Department of Anatomy, University of Massachusetts Medical School, Worcester, MA 01605.

Mutations at the shi (chromosome 18) and jp (X-chromosome) loci each produce CNS hypomyelination with complex, but clearly distinguishable, biochemical and morphological phenotypes. Double mutant mice which carry both shi and jp mutations (shi*jp) have a phenotype with features of each single mutant disease, but in milder form. The shi mutation has a presumed allele, shi$^{mld}$. On the B6C3 background, shi$^{mld}$ phenotypically resembles shi but has more major dense line in its myelin. The jp mutation also has a presumed allele, jp$^{msd}$, which resembles jp but has more numerous myelin sheaths. We have sought more information about these two genetic loci and their interactions by comparing the morphological phenotype of shi$^{mld}$*jp$^{msd}$ double mutant mice with the phenotype of shi*jp. Three successive generations of crossing proven, genetically marked B6C3 Tajp$^{msd}$/+ females to B6C3 shi$^{mld}$/shi$^{mld}$ males produced marked, doubly-affected B6C3 shi$^{mld}$/shi$^{mld}$*Tajp$^{msd}$/Y males as well as crossover B6C3 shi$^{mld}$/shi$^{mld}$*Ta+/Y controls. In the putative double mutant shi$^{mld}$*jp$^{msd}$ mice, myelin was reduced in amount in the cerebellum, optic nerve, and forebrain at postnatal days 21-24 relative to either shi$^{mld}$ or jp$^{msd}$. The few sheaths present were thinner than are normally found in jp$^{msd}$. As in shi$^{mld}$, myelin surrounded only part of the axon circumference in many cases; sheaths included cytoplasm between lamellae, and had an abnormally dense intraperiod line. All sheaths examined thus far have lacked any major dense line. By contrast, B6C3 shi$^{mld}$ CNS myelin clearly shows some major dense line formation at this age. Oligodendrocytes were reduced in number relative to shi$^{mld}$, and the parallel arrays of oligodendrocyte microprocesses so prominent in shi$^{mld}$ were not obvious in the available sample. The lipid-filled cells characteristic of jp$^{msd}$ white matter were numerous. Thus the jp$^{msd}$ mutation makes some, although not all morphological features of the shi$^{mld}$ phenotype more severe, and the shi$^{mld}$ mutation tends to behave similarly toward the jp$^{msd}$ phenotype. The morphological phenotype of B6C3 shi$^{mld}$*jp$^{msd}$ double mutant animals therefore suggests an intergenic interaction which is either additive or synergistic depending upon the characteristics considered. This conclusion differs sharply from results obtained in B6C3 shi*jp mice, in which the two mutant genes exert partial reciprocal suppression.
Supported by NIH grant NS11425 (Javits Award).

316.6 MOLECULAR BASIS FOR HETEROSIS FOR BRAIN MYELIN CONTENT IN MICE. R. Miskimins*, H. Ebato*. T.N. Seyfried and R.K. Yu. Dept. of Neurology, Yale University School of Medicine, New Haven, CT 06510

We have shown previously that brain myelin content was significantly higher in the $F_1$ hybrid (B6 x D2) than in either C57BL/6J (B6) or DBA/2J (D2) parental strain of mice (Ebato, H., Seyfried, T.N. and Yu, R.K. J. Neurochem., 40:440, 1983). This represents a classical example of heterosis, that is, the $F_1$ hybrid possesses a trait in excess of either parent. This heterosis for myelin content involved several parameters including amount of myelin isolated, concentrations of brain myelin protein, cerebroside, $G_{M1}$ ganglioside and the activities of CNPase, 5'-nucleotidase and carbonic anhydrase. In order to determine the molecular level at which this heterotic effect was regulated we performed the following experiments.

Poly(A)$^+$ messenger RNA (mRNA) was isolated from the brains of B6, D2 and $F_1$ (B6 x D2) hybrid mice at 16-17 days of age. The mRNA was translated in vitro in a rabbit reticulocyte lysate system. A significant heterotic effect was seen for the production of myelin basic protein (MBP) by the mRNA from the brains of the $F_1$ hybrid mice as compared with MBP production of both parental strains. The relative distribution of the MBP's produced was the same for all three strains. The amounts of total polysomal RNA, recoverable poly(A)$^+$ RNA and the rate of incorporation of labelled amino acid were not significantly different among the three strains of mice. Northern blot analysis of polysomal poly(A)$^+$ RNA with a cDNA probe for MBP showed that the amount of mRNA coding for MBP present in the polysomes was significantly greater in the $F_1$ mice. This analysis showed a single band in all three strains. Northern blot analysis of poly(A)$^+$ RNA from the total brain again showed $F_1$ to contain the greatest amount of RNA coding for MBP. The blots from this RNA showed a second band of greater length that was present in a much smaller amount. Taken together we believe these results imply that heterosis for brain myelin content in the $F_1$ hybrid mice may be regulated at the transcriptional level.

316.7 **ISOLATION OF A POSSIBLE NEURONAL NICOTINIC ACHR ALPHA SUBUNIT cDNA CLONE FROM THE RAT PC12 CELL LINE**

by Jim Boulter, Gary Martin, Karen Evans, Dan Goldman, Pam Mason Doug Treco, Steve Heinemann and Jim Patrick. Molecular Neurobiology Laboratory, The Salk Institute, San Diego, CA 92138

Advances in understanding the molecular biology of the muscle nicotinic acetylcholine receptor (nAChR) have been facilitated by recombinant DNA methodologies. We employed a similar approach to the study of the nerve nicotinic acetylcholine receptor by isolating cDNA clones coding for this protein. Using mouse muscle nAChR cDNA probes and Northern blot hybridization analysis, we have shown that the mouse α-subunit cDNA hybridizes to mRNA sequences in non-muscle tissue such as brain, adrenal medulla, and the clonal rat sympathetic nerve cell line PC12. We constructed a cDNA library using mRNA from PC12 cells and $\lambda gt_{10}$ as vector. The library was screened with the mouse nAChR α-subunit cDNA and three independent, overlaping clones were isolated from the 950,000 plaques examined. The longest of the three clones, λPC12α48, contains insert DNA of approximately 2100 base pairs. Partial deduced amino acid sequence of this clone reveals homology with cloned nAChR α-subunits from calf, human, mouse and Torpedo of approximately 50% while, in the region compared, the calf, human and mouse muscle α-subunit homology is approximately 95%. A comparison of the deduced amino acid sequence of the PC12α48 clone with similar regions in mouse γ, δ and calf β cDNA clones yields homologies of 21%, 30% and 33% respectively. S1 nuclease analysis of heteroduplexes formed between PC12α48 cDNA and mRNA isolated from PC12 cells or denervated rat diaphragm show that the PC12α48 cDNA protects mRNA species from PC12 (non-muscle nAChR) but not from rat diaphragm (muscle nAChR). Radiolabeled PC12α48 cDNA hybridizes to poly $(A)^+$ selected RNA from calf adrenal medulla- but not cortex, and to rat adrenal gland, rat hypothalamus and, at lower stringency, rat cerebellum and hippocampus. Finally, Southern blot analyses of mouse genomic DNA demonstrates that the PC12α48 cDNA clone is the product of a gene distinct from the gene encoding the muscle nAChR α-subunit.

316.8 MOLECULAR CLONING OF AN OLFACTORY NEURON SPECIFIC PROTEIN. K.E. Rogers*, M. Grillo*, M. Poonian*, U. Gubler* and F. L. Margolis. Roche Inst. Molec. Biol. and Dept. Molecular Genetics, Roche Res. Ctr., Nutley, N. J. 07110.

Olfactory marker protein (OMP) is a neuron-specific protein of Mr=18,500, located exclusively in olfactory chemoreceptor cells. It is synthesized only by mature neurons and not by their progenitor cells and is thus developmentally regulated. Although the biochemical properties of OMP have been extensively characterized, its function remains unknown. Therefore, we have recently begun to examine OMP at a more fundamental molecular level.

Prior to cloning OMP-mRNA was characterized in this laboratory. Using *in vitro* translation and immunoprecipitation, we demonstrated that OMP is synthesized directly from rat olfactory mucosal poly(A)$^+$RNA without the formation of a larger polypeptide precursor. In addition, it was demonstrated that the size of OMP mRNA is 2500-3300 nucleotides. This exceeds the minimum coding requirement for OMP(500 bases) by at least a factor of five. Using sucrose gradient fractionated OMP-mRNA as a template, double stranded complementary DNA (cDNA) was synthesized. The cDNA was introduced into the *Eco RV* site of the plasmid pMG 5. *E. coli* DH1 was transformed with the insert-containing plasmids, creating a partial olfactory mucosal cDNA library. Colonies were screened for OMP inserts using a synthetic heptadecameric oligodeoxynucleotide probe with a degeneracy of sixteen which had been predicted from the known amino acid sequence of OMP. Previously, this probe had been shown by Northern blot analysis to hybridize exclusively to mRNA containing the OMP message. Three OMP-positive colonies were detected out of 10,000 colonies originally screened. Restriction analysis of the plasmids contained in these clones with *Eco* R1 and *Hinc* II endonucleases demonstrated that the insert sizes were 2100-2300 nucleotides. Excision of the inserts with *Bam HI* indicated the presence of an additional *Bam* H1 restriction site in the sequences of all three clones Southern analysis of the *Bam HI* restriction fragments with the synthetic oligodeoxynucleotide probe indicated that the probe hybridized only to the larger 1350 nucleotide fragment. Translation of hybrid selected olfactory mucosal poly(A)$^+$RNA followed by immunoprecipitation of OMP was used as an additional confirmation of the identity of these clones. The linearized insert-containing plasmids, but not unmodified pMG 5 or pBR 322 plasmids, were able to select translatable OMP-mRNA. The nucleotide sequences of these OMP cDNA clones are now being determined. The availability of these clones should facilitate our attempts to understand the mechanisms involved in regulating the cell specific expression of this unique protein.

316.9 **ISOLATION OF cDNA CLONES FOR VASOPRESSIN AND OXYTOCIN FROM AN EXPRESSION LIBRARY OF RAT HYPOTHALOAMIC POLY(A)-RNA.** Norbert E. Kremer and Jeffrey F. McKelvy, Dept. of Neurobiology and Behavior, SUNY, Stony Brook, NY 11794.

In order to study the regulation of gene expression of the neurohypophysial peptides, vasopressin and oxytocin, it is desirable to have hybridization probes of a variety of sizes and sequence specificities. We have isolated cloned cDNAs for the precursors of these peptides from the rat, since in this animal both the neuroendocrine and CNS functions of these peptides may be studied. A cDNA expression library in the phage vector lambda gt11 was made from rat hypothalamic poly(A) RNA and kindly provided by Drs. R. Goodman and G. Mandel. Double-stranded cDNA was made through the use of RNAse H and ligated to the vector by means of EcoRI linkers; before amplification the library contained an estimated two million independent clones.

Clones were initially selected for expression of neurophysin immunoreactivity using the anti-rat neurophysin antibody RN-4 of Dr. A. Robinson and goat anti-rabbit/alkaline phosphatase conjugate. Positives in the initial screening arose at a rate of about one per twenty thousand plaques. Clones were plaque-purified and then further screened with monoclonal antibodies to rat vasopressin and oxytocin precursors (kindly provided by Dr. H. Gainer). These monoclonal antibodies allowed classification of the expression-positivie clones as vasopressin-like or oxytocin-like. Further characterization of the cDNA inserts is being made using synthetic oligonucleotide probes made according to the cDNA sequence predicted from the published rat genomic sequences.

When the cDNAs so derived are labelled with $^{32}P$, and used to probe RNA blots of rat hypothalamic RNA, RNA species of molecular weights appropriate for vasopressin and oxytocin may be identified.

These clones will be useful for the study of the physiological regulation of vasopressin and oxytocin. Furthermore, the precursor genes, or parts thereof, when cloned into expression vectors, may be used to program the synthesis of large amounts of defined portions of the precursors. These synthetic precursor molecules will be useful in the study of neuropeptide processing enzymes in vivo and in vitro.

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316.10 ALKALINE RIBONUCLEASE IS PRESENT IN AXOPLASM OF THE GIANT AXON OF THE SQUID. N.A. Ingoglia, Dept. of Physiology, New Jersey Medical School, Newark, NJ 07103

Alkaline ribonuclease is a cytosolic enzyme capable of hydrolyzing cellular RNA. Its activity has been shown to be inhibited by a 50-60 kD protein which is linked to it by sulfhydryl bonds. The removal of this inhibitory protein by sulfhydryl blocking agents results in activation of the enzyme, and subsequent hydrolysis of RNA. It appears that this enzyme plays a role in the control of protein synthesis by regulating the availability of RNA. Alkaline ribonuclease is present in human brains and the activity of the enzyme inhibitor complex has recently been shown to be altered in patients who have succumbed to Alzheimer's disease. (Sajdel-Sulkowska and Marotta, Science 225:947, 1984).

In the present experiments we have investigated the possibility that the enzyme is present in axoplasm. Since axonal RNA is at least 85% transfer RNA (Lasek et al., Nature 244:118, 1973) and transfer RNA in axons appears to act as an amino acid donor in posttranslational protein modification (Ingoglia et al., J. Neurosci. 3, 12:2563, 1983), the implication of finding alkaline ribonuclease in axoplasm would be that its function might be to regulate protein modification in addition to, or rather than, synthesis of protein. Axoplasm was obtained from the giant axon of the squid and aliquots of the 80kxg supernatant were incubated with or without p-chloromercuribenzoate (PCMB), a sulfhydryl bridge blocking agent, and $^3H$-RNA which served as substrate. Alkaline ribonuclease activity was monitored by measuring the hydrolysis (liberation into a TCA soluble fraction) of $^3H$-RNA. Reactions were run for 1 hr at 37°C and compared with 0°C, unreacted controls or controls incubated without biological material. Alkaline ribonuclease activity (without PCMB) was 4 to 5 times greater than 0°C controls and twice that of similar reactions run in the 80kxg supernatant fractions of rat brain homogenates. The data suggest the presence of active alkaline ribonuclease in squid axoplasm. While the addition of PCMB caused a five fold increase in alkaline ribonuclease activity in rat brain, its addition to axoplasm caused a 55% decrease in alkaline ribonuclease activity.

The results indicate that axoplasm contains alkaline ribonuclease which may play a role in the regulation of tRNA dependent protein modification by amino acids, but contrary to findings in higher vetrebrates, its activity does not appear to be regulated by a protein inhibitor. Supported by NINCDS grant NS19148 from NIH.

316.11 POSTTRANSLATIONAL INCORPORATION OF ARGININE AND LYSINE INTO RAT BRAIN SOLUBLE PROTEINS IN VITRO. G. Chakraborty* and N.A. Ingoglia. Department of Physiology and Neuroscience, New Jersey Medical School, Newark, NJ 07103

The posttranslational modification (PTM) of rat brain proteins has been reported to involve a tRNA dependent addition of Arg but not Lys or other amino acids to endogenous substrates (Barra et al., 1973, J. Neurochem. 20, 97). These experiments required the removal of molecules of less than 5kD for expression of the reaction. In similar experiments (except that molecules of less than 125kD were removed), we have demonstrated the PTM of axonal proteins by Arg and Lys (as well as other amino acids) (Ingoglia et al, 1984, Advances in Neurochemistry 6, 119). In the present experiments we have investigated the possibility that by changing the molecular exclusion properties of a rat brain supernatant preparation, that not only Arg, but Lys could also be incorporated into rat brain proteins.

No significant incorporation of $^{3}$H-amino acids into proteins occured when the unfractionated soluble supernatant was incubated with $^{3}$H-amino acids and an appropriate reaction mixture. However, when the soluble supernatant was fractionated by Sephadex G25-40 chromatography, the void volume (containing molecules 5kD) was able to incorporate $^{3}$H-Arg (264.99 $\pm$ 40.82 DPM/ug Protein) but not $^{3}$H-Lys (2.15 $\pm$ 0.52 DPM/ug Protein) into endogenous proteins. These data confirm the findings of Barra et al (1973). When the soluble supernatant was fractionated by Sephacryl S-200 chromatography, comparable amounts of $^{3}$H-Arg were incorporated into proteins (172.13 $\pm$ 44.25 DPM/ug Protein) but $^{3}$H-Lys was now also incorporated into proteins (32.02 $\pm$ 10.93 DPM/ug Protein).

SDS-PAGE of $^{3}$H-Arg or $^{3}$H-Lys modified proteins revealed similar labelling patterns of proteins of approximately 70, 53, 38, 22 and 12-18kD. The addition of the inactive supernatant to the active G25-40 or S-200 void volume to a final concentration of 10% resulted in the inhibition of both reactions by 50% (Arg) or 85% (Lys).

The data suggest the presence of separate inhibitory substances in rat brain (for Arg 5kD; for Lys 125kD) which regulate their posttranslational incorporation into endogenous proteins. (Supported by NINCDS Grant NS-19148 from NIH.)

316.12 EXPRESSION OF GENES ENCODING TYPES I AND IV COLLAGEN DURING MYOGENESIS OF SKELETAL MUSCLE CELLS IN CULTURE. J.S. Rao,* W.J. Lindlbad,** and Barry W. Festoff Neurobiology Research Lab, VAMC, Kansas City, MO. and Dept. of Neurology, University of Kansas Medical Center, Kansas City, KS., ** Dept. of Surgery, Medical College of Virginia, Richmond VA. (SPON: R. Miller)

During myogenesis, skeletal muscle cells in culture produce collagen types I and IV. This was examined at the molecular level by cell-free translation and dot hybridization techniques. Total RNA was extracted from skeltal muscle cells (from day 3 myoblasts and day 10 myotubes) during myogenesis and translated in a cell-free translation system. The translation products were characterized on SDS-PAGE before and after collagenase digestion. In this manner, the translation of both procollagen types I and IV was demonstrated. To quantitate the amount of these procollagen types during myogenesis, extracted RNA was hybridized to the following cloned cDNA probes: P$\alpha$1R1 for pro-$\alpha$1 mRNA; P$\alpha$2R2, for pro-$\alpha$2(I) mRNA and pPE41 for pro$\alpha$1(IV) mRNA. When compared to myoblasts, myotube cultures had decreased levels of pro$\alpha$1(I) but increased pro$\alpha$2(I) as determined by solid support hybridization with P$\alpha$1R1 and P$\alpha$2R2 respectively. Myotube cultures also had increased levels of pro 1(IV) as determined by dot hybridization with pPE41. These results demonstrate that synthesis of interstitial collagen (type I) is decreased while synthesis of basement membrane collagen (type IV) is increased during myogenesis.

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## ACETYLCHOLINE: RECEPTORS

317.1 PRESYNAPTIC CONTROL OF 3H-ACETYLCHOLINE (3H-ACH) RELEASE FROM CORTICAL SLICES OF YOUNG AND AGED FISHER 344 RATS. C. J. Spencer*, R. D. Schwarz and T. A. Pugsley*. (SPON: R. F. Bruns). Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48105.

Considerable debate exists in the literature about the effects of aging on the function of central cholinergic neurons. There are conflicting reports on changes in the activity of both the synthetic and degradative enzymes for ACh, and on changes in both nerve terminal choline uptake and ACh release. In contrast, most studies have confirmed an age-related decrease in the number of muscarinic receptors while their affinity is not affected. The present study examines aspects of presynaptic control of $^{3}$H-ACh release from slices of cerebral cortex in young (3-6 mos) compared to aged (24-26 mos) male Fisher 344 rats.

The cortex was removed and cut into 0.3 x 0.3 mm slices. These were incubated in Krebs-Ringer Hepes buffered medium, pH 7.2 (KRH) with 0.01 $\mu$M $^{3}$H-choline for 15 min at 37$^{o}$ C. After washing, the slices were again incubated for 15 min in KRH medium with the addition of a depolarizing agent; muscarinic agonists or antagonists were added at this time. Release was terminated by cooling and rapid separation of slices from medium by centrifugation. $^{3}$H was determined in both fractions by scintillation spectrometry.

Spontaneous release of $^{3}$H was the same for both age groups. With increasing concentrations of $K^{+}$ (10-40 mM) or veratridine (0.1-100 $\mu$M) the release of $^{3}$H-ACh increased very similarly in both young and aged. Oxotremorine (0.01-100 $\mu$M) decreased $K^{+}$-stimulated $^{3}$H-ACh release in a concentration-dependent manner in both groups; this decrease was reversed by atropine. There was little variation between young rats in response to oxotremorine. In contrast the oxotremorine effect on the aged rats varied markedly from individual to individual. However, a comparison of the data pooled within each age group showed no difference between young and aged; a maximal decrease of 50% occurred at approximately 10 $\mu$M for both. Since presynaptic receptors may control $Ca^{++}$ entry, the effect of $Ca^{++}$ on $K^{+}$-stimulated $^{3}$H-ACh release was examined. The addition of increasing concentrations of $Ca^{++}$ (0.13-2.6 mM) resulted in increasing $K^{+}$-stimulated $^{3}$H-ACh release. Young animals showed less variation than aged animals yet the pooled results revealed no significant difference between the two age groups. In addition, choline acetyltransferase activity in the cortex of both young and aged rats is currently being examined.

In general, the presynaptic control of $^{3}$H-ACh release by muscarinic agonists and the utilization of extracellular $Ca^{++}$ appear not to be altered consistently by aging. There may indeed be an effect of age on mechanisms controlling ACh release, but it may be specific to each individual studied. Rodents, like humans, appear to age and acquire cholinergic deficits at different rates.

317.2 PHARMACOLOGY OF MUSCARINIC RECEPTORS CONTROLLING ACH RELEASE IN THE RAT IRIS. T. Mattio*, E. Giacobini and V. Hoban*. Department of Pharmacology, Southern Illinois University School of Medicine, Springfield, IL 62708 USA

The release of acetylcholine (ACh), both in central and peripheral nerve tissues, seems to be modulated by a presynaptic muscarinic receptor. In the albino rat iris, which contains a dense cholinergic plexus, we have demonstrated the presence of a muscarinic autoreceptor. The electrically stimulated release of ACh (50 Hz, 20 mA, 5 ms biphasic square wave) was increased in the presence of muscarinic antagonists. Pirenzepine increased ACh release in a dose dependent manner from $10^{-3}$ M (by 90%) to $10^{-7}$ M (by 27%). Scopolamine also showed a dose-dependent increase in ACh release. At $10^{-4}$ M and $10^{-5}$ M scopolamine increased ACh release by 88% and 41%, respectively. Atropine increased ACh release from $10^{-3}$ M (by 70%) to $10^{-7}$ M (by 55%). Oxotremorine decreased the stimulated release of ACh at $10^{-3}$ M and $10^{-5}$ M. Both pirenzepine and atropine antagonized this effect. 2-Aminopyridine, 3-aminopyridine and 3,4-diaminopyridine were without effect on ACh release, however, 4-aminopyridine increased ACh release by 55% at $10^{-3}$ M. Hemicholinium ($10^{-3}$ M) increased the stimulated overflow of ACh in the iris. These results demonstrate the effects of muscarinic antagonists and agonist on the release of ACh. This release is controlled by a presynaptic muscarinic receptor which when stimulated decreases ACh release and when blocked increase ACh release. In the iris the aminopyridines are not as effective as has been found at neuromuscular junctions. Hemicholinium which prevents the reuptake of choline after ACh hydrolysis shows a marked increase in ACh overflow after electrical stimulation. This overflow is most probably due to the effect on uptake and not to an effect on the muscarinic autoreceptor. The rat iris offers several advantages in studies of actions of drugs on muscarinic receptors and ACh release. (Supported by grant AFOSR-83-0051 to E.G.)

317.3 FUNCTIONAL AND BIOCHEMICAL STUDIES OF THE PUTATIVE $M_1$ SELECTIVE MUSCARINIC AGONIST MCN-A-343 [3-(m-CHLOROPHENYLCARBAMOYLOXY)-2-BUTYNYLTRIMETHYLAMMONIUM] CHLORIDE. M. Watson, T.W. Vickroy, H.I. Yamamura and W.R. Roeske. Departments of Pharmacology and Internal Medicine, University of Arizona Medical School, Tucson, AZ 85724.

McN-A-343 stimulates ganglionic muscarinic receptors, thus increasing blood pressure and heart rate. It has been suggested that McN-A-343 produces a selective stimulatory muscarinic effect upon sympathetic ganglia which overrides weaker muscarinic effects in the heart and vasculature. McN-A-343 has also been proposed to selectively interact with muscarinic receptors (MR) which show high affinity for pirenzepine (PZ) in radioligand binding studies. Thus, we have investigated the properties of McN-A-343 in rat cerebral cortical and cardiac membrane preparations and intact cell preparations. The formation of cardiac cyclic AMP (cAMP) is regulated by an inversely coupled complex between MR, β-adrenergic receptors (BAR), adenylate cyclase and other membrane constituents. In adult rat intact cardiomyocytes attached to culture plates, the BAR agonist (-)isoproterenol (ISO) produces a potent ($EC_{50}$=22 nM) and rapid (15s) increase (10x) in endogenous cAMP levels. The effect of ISO is completely reversed by the cholinergic agonists acetylcholine (ACH) and carbamylcholine (CAR) in this system. This reversal is blocked by 3OnM atropine (ATR). McN-A-343, while its $K_{act}$ (concentration producing 1/2 maximal response) is 19nM, has little efficacy, showing 1/10 the effect of ACH. Inhibition studies were conducted to observe the binding and regulation of McN-A-343 to putative MR subtypes in cerebral cortical and cardiac membranes labeled with the classical muscarinic antagonist [$^3$H](-)quinuclidinyl benzilate ([$^3$H](-)QNB), the nonclassical putative $M_1$ MR selective antagonist [$^3$H]pirenzepine ([$^3$H]PZ) and the potent MR agonist [$^3$H](+)cismethyldioxolane ([$^3$H](+)CD). Binding studies in 10 mM Na-K, 50 mM Na-K and modified Krebs phosphate buffer at 25°C in the presence and absence of the relatively non-hydrolyzable GTP analog, guanyl-5'-yl imidodiphosphate [Gpp(NH)p] show that McN-A-343 has a lower affinity for MR in a higher ionic strength buffer and also when Gpp(NH)p is present. McN-A-343 is shifted to about 5x lower affinity by Gpp(NH)p in the heart. Although McN-A-343 data is better fit to a 2-site model in [$^3$H](-)QNB labeled and [$^3$H](+)CD labeled cerebral cortical membranes and in [$^3$H](-)QNB labeled intact neuronal cells, only 1-site was seen versus [$^3$H]PZ labeled membranes. An extremely high affinity McN-A-343 site (2 nM) seen in [$^3$H](+)CD labeled and [$^3$H](-)QNB labeled cerebral cortical homogenates was also seen by direct binding. McN-A-343 showed 2-sites in cardiac membranes versus [$^3$H](-)QNB, but not versus [$^3$H](+)CD. Although the relative selectivity of McN-A-343 varied with assay conditions, it was up to 4 fold selective for cerebral cortical MR, as compared to cardiac MR. Thus, McN-A-343 acts as a weak partial agonist and is somewhat selective for putative $M_1$ MR sites in the cerebral cortex, in contrast to other agonists such as ACH and CAR.

317.4 TWO PHYSIOLOGICALLY AND PHARMACOLOGICALLY DISTINCT SUBCLASSES OF MUSCARINIC RESPONSES IN CEREBRAL CORTICAL NEURONS. D.A. McCormick and D.A. Prince. Dept. of Neurology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

Application of acetylcholine (ACh) to presumed pyramidal neurons in layers II-III of the anterior cingulate cortical slice evokes a short latency inhibition due to activation of GABAergic interneurons, followed by a delayed direct prolonged voltage-dependent depolarization presumably due to decrease in a $K^+$ conductance ("M-current"). We characterized the pharmacological properties of these ACh responses by comparing the actions of various drugs on the cholinergic inhibition and slow excitation of pyramidal neurons. One or 10 μM atropine or scopolamine completely blocked all responses to ACh, while 0.1 μM atropine, 10 μM hexamethonium, and 10-100 μM dihydro-beta-erythroidine did not; indicating that these actions of ACh are mediated through muscarinic receptors. Pirenzepine (PZ) distinguished between these receptors in a dose-dependent manner: 1 μM PZ diminished, while 10 μM PZ blocked the slow excitatory response (Fig. 1).

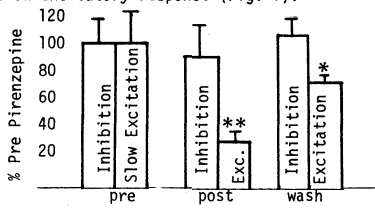


Fig. 1 Effect of PZ (10 μM) on inhibitory and slow excitatory response of pyramidal neurons to ACh. Pre, post and wash data from same neurons (n=6). Mean +/- sem. ** - $p<0.01$, * - $p<0.05$.

The inhibitory response was unaffected by these doses, but was diminished or blocked by 50 μM PZ. Thus PZ was 5-50x more effective in blocking the slow depolarizing vs. the hyperpolarizing response. These responses also differed in their sensitivities to various cholinergic agonists. Carbachol, metacholine, propionylcholine, and oxotremorine-M evoked both inhibitory and slow excitatory responses, while pilocarpine, suberyldicholine, and DL-muscarine were much more effective in activating slow excitation than the inhibition. Oxotremorine elicited the inhibitory response only but blocked the slow excitatory response evoked by ACh.

These results indicate that ACh has two muscarinic actions on cortical neurons: 1) a slow excitation of cortical pyramidal cells produced by presumed decrease in the "M-current" mediated via high affinity PZ-muscarinic receptors; and 2) a rapid excitation of GABAergic interneurons probably produced by an increase in cation conductance mediated via low affinity PZ-muscarinic receptors.

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317.5 INTERACTION BETWEEN MUSCARINIC ACETYLCHOLINE RECEPTOR AND NEUROPEPTIDES IN THE RAT HIPPOCAMPUS. R. Miyoshi*, S. Kito, K. Mizuno* and H. Matsubayashi*. Third Department of Internal Medicine, Hiroshima University School of Medicine, Hiroshima 734, Japan.

The cholinergic cell bodies located at the medial septal nucleus and nucleus of the diagonal band, project via the fimbria/fornix to innervate the pyramidal cells of the hippocampus. From histochemical studies, it seems clear that nerve cell bodies containing vasoactive intestinal polypeptide (VIP), cholecystokinin octapeptide (CCK-8), somatostatin and enkephalins are present in the hippocampus. As for substance P, it is reported that substance P positive perikaria are not present within the hippocampus, but its receptors have been known to exist substantially. This is also the case with vasopressin, a memory-related peptide. The authors observed interaction between muscarinic acetylcholine receptor (mAchR) and somatostatin in the rat hippocampus through binding experiments, and presented the results at the last Annual Meeting of Society for Neuroscience. It was noticed that somatostatin reduced the affinity of mAchR agonist binding, and this effect was dose-dependent and limited to agonist binding. In this paper, the authors studied interaction between mAchR and other peptides in the rat hippocampus. Used peptides were VIP, CCK, enkephalins, substance P and vasopressin. mAchR agonist binding experiments were performed with use of $^3$H- oxotremorine-M-acetate ($^3$H-oxo-M) as radioactive ligand and acetylcholine of $10^{-4}$ M was used as non-radioactive ligand. It is accepted by most pharmacologists that there are two or three subtypes in mAchR agonist binding sites. In our experiments, the presence of two components of mAchR agonist binding sites was observed when Krebs Henseleit solution was used, whereas a single binding site was obtained by switching the buffer to Na-K phosphate solution. $P_2$ fraction of the rat hippocampus was incubated in Na-K phosphate buffer at 30° C for 8 min. Scatchard analysis of saturation experiments revealed that both VIP and vasopressin decreased the affinity of $^3$H-oxo-M binding like somatostatin. These peptides did not influence to a $B_{max}$ value of $^3$H-oxo-M binding sites. In the contrary, snbstance P decreased a $B_{max}$ value with a Kd value unchanged. The effect of these peptides was dose-dependent. It is assumed that these peptides are acting as neuromodurators at the either presynaptic or postsynaptic level.

317.6 RETINAL ALTERATIONS OF CHOLINERGIC PARAMETERS FOLLOWING INTRAOCULAR INJECTIONS OF THE CHOLINE MUSTARD AF64A. C. Estrada, D. Triguero* and P. Gómez-Ramos. Departamento de Fisiología y Departamento de Morfología, Facultad de Medicina, Universidad Autónoma, 28029 Madrid. SPAIN.

The compound ethylcholine mustard aziridinium ion (AF64A) is thought to have a selective toxic effect on cholinergic neurons. In the present report morphologic and biochemical changes induced by AF64A were analyzed in the rat retina. Unilateral intraocular injections (5 μl) of either 10, 25 or 50 nmol were performed in adult rats. The animals were killed 1, 7 or 30 days after the injections, and the retinas were excised and examined under light microscopy. Choline acetyltransferase (ChAT) activity was evaluated both in control and experimental retinas. Muscarinic receptor binding sites were determined by measuring the binding of tritiated quinuclidinylbenzilate ($^3$H-QNB) to the 27,000 x g pellet of retina homogenates.

One day after injections of 10 nmol AF64A, ChAT activity was decreased to 33±6% of the control values. This reduction persisted one week (33±6%) and one month (37±10%) later. The histological study one week after the injections showed cytoplasmic swelling in some neurons located in the innermost part of the Inner Nuclear Layer (INL) and occasionally in the Ganglion Cell Layer (GCL), where the retinal cholinergic neurons are thought to be localized. One week after injections of 25 and 50 nmol only residual ChAT activities of 11±1% and 2.2±0.6% of the control values were observed in the experimental retinas. The examination of the later group of retinas showed a great reduction of the retinal thickness together with a disappearance of most cells in the Outer Nuclear Layer (ONL). The Outer Plexiform Layer (OPL) could not be distinguished. The neurons of the INL and GCL, although normal in appearance, seemed to be reduced in number.

The amount of muscarinic receptor binding sites showed no alteration at any dose or any time after the AF64A administration.

The results observed after the administration of the lowest dose used (10 nmol) are in accordance with a selective toxic effect on cholinergic neurons although higher doses produce a more generalized and nonspecific effect.

317.7 CELLULAR LOCALIZATION OF $M_1$ AND $M_2$ MUSCARINIC ACETYLCHOLINE RECEPTORS IN CINGULATE CORTEX. Brent A. Vogt. Depts. of Anatomy and Physiology, Boston University School of Medicine, Boston, MA 02118

There are regional variations in the densities of $M_1$ and $M_2$ muscarinic acetylcholine receptors in the central nervous system. For example, $M_1$ sites are concentrated in the substantia gelatinosa, cerebral cortex and caudate-putamen, while large numbers of $M_2$ sites are in the hypoglossal nucleus, cerebellum and ventral horn. There is, however, no information available regarding the cellular locations of these receptor subtypes in the CNS. In this study ablation/degeneration techniques have been used to identify the neuronal and afferent axonal positions of these receptors.

Ablations were placed in rats either in the anterior thalamic nuclei (ATN) via thermocoagulation or in area 29 of posterior cingulate cortex with the neurotoxin ibotenic acid. Two weeks postoperatively the animals were sacrificed, blocks removed and frozen and 16 μm thick sections cut with a cryostat. Alternate sections were then incubated in either $^3$H-propylbenzilylcholine mustard (PrBCM, 2.4 nM) which is a muscarinic antagonist that binds to both $M_1$ and $M_2$ receptors or $^3$H-pirenzepine (PZ, 15 nM) which binds selectively to $M_1$ sites. Some sections were coincubated in atropine (1 μM) to assess nonspecific binding. Standard autoradiographic procedures were employed and grain densities per 2500 $\mu m^2$ quantified with a high resolution automated grain counting system.

Normal, specific binding of PrBCM is heterogeneous with peak binding in layers Ia and IV. This binding is reduced following ATN lesions by 30% and 15%, respectively. In contrast, PZ binding is essentially homogeneous throughout the cortex, although it is lowest in layer Ia and highest in layer Ic (275 vs 354 grains/2500 $\mu m^2$). Ablations of the ATN fail to alter this latter binding pattern. However, specific binding of PZ is reduced by almost 80% following neuronal degeneration produced by ibotenic acid injections. In these same cases peaks in PrBCM binding in layers Ia and IV remain following ibotenic ablations as does approximately 30% of the binding in all other layers. It is possible that much of this latter binding is associated with $M_2$ receptors.

The following conclusions are drawn from these observations. First, muscarinic receptors on ATN axons may be of the $M_2$ subtype because normal PZ binding is lower in those layers which receive most thalamic input and no change occurs after thalamic lesions. Second, neuronal dendritic receptors are of the $M_1$ subtype, since radical reductions in PZ binding follow neuron removal. Thus, ligands for the $M_1$ and $M_2$ receptors may be used as differential probes to activate or block acetylcholine receptors in dendritic or axonal positions.

Supported by NINCDS grant 18745.

317.8 THE INTERACTION OF GALLAMINE WITH RAT BRAIN $M_1$ MUSCARINIC RECEPTORS, STUDIED BY COMPETITION BINDING WITH $^3$H-PIRENZEPINE. R.E. Burke. Dept. of Neurology, Columbia University, New York, N.Y. 10032.

Pirenzepine (Pir) distinguishes subclasses of muscarinic receptor, M1 and M2, for which it has high and low affinity, respectively. Gallamine has muscarinic antagonist properties and , like Pir, it distinguishes subclasses (Ellis & Hoss, 1982; Stockton et al, 1983; Dunlap & Brown, 1983). Gallamine binds with high affinity in tissues with mainly M2 sites (hindbrain, heart), and lower affinity in tissues with mainly M1 sites (forebrain). Thus, gallamine-recognized heterogeneity appears to parallel that defined by Pir. However, the binding of gallamine to the M1 and M2 subtypes, defined by Pir binding, has not been characterized at either site in binding experiments which examine one or the other subtype exclusively. Because a filtration assay for $^3$H-Pir reveals linear Scatchard plots with a KD equal to that for the M1 site (Watson et al, 1983), such binding represents an interaction primarily with the M1 site.

$^3$H-Pir binding was performed as described by Watson et al. Study of association rates showed that equilibrium was achieved at ½ hr. incubation at 25°C in the presence of 100 nM gallamine. $^3$H-Pir dissociation rate was not affected by 30 nM gallamine. Competition binding studies showed complete inhibition of $^3$H-Pir binding at gallamine ≥ 1000nM. Competition binding studies with cortical membrane preparations, analyzed with the LIGAND program, were best described with a one-site model with Ki= 24 nM. Similarly, studies with striatal and hippocampal membranes were fit with a one site model, with Ki= 28 and 23 nM, respectively. In medulla-pons memebrane preparations, gallamine had an $IC_{50}$ of 22 nM. A Schild plot analysis of the effect of gallamine on $^3$H-Pir KD, determined by Scatchard plots, was consistent with a competitive interaction, with Ki= 28 nM.

We conclude that gallamine interacts with the M1 site in competitive fashion, and that gallamine recognizes only one site within the M1 muscarinic receptor subpopulation.

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317.9 THE EFFECTS OF SPIROMUSTINE ON THE CHOLINERGIC SYSTEM IN THE RAT BRAIN. N.S. Nadi* and T.G. Staunton* (Spon: R.J. Porter). Medical Neurology Branch and Surgical Neurology Branch, NINCDS, Bethesda, MD 20205

Acetylcholine plays an important role in the control of motor activity, arousal, learning and memory processes in the central nervous system (CNS). Recently, during the clinical trials of a potential anti-brain tumor mustard spiromustine (SHM), it was noted that patients developed episodes of confusion, obtundation and hallucinations which were reversed by physostigmine (Staunton, T.G. et al. Ann of Neurol, in press). These observations indicated that some of the effects of SHM were mediated through the cholinergic system. The effect of SHM on the CNS of rats was investigated following an intravenous injection of 2.5 mg/kg of SHM. Three brain areas, the frontal cortex, the basal ganglia and the hippocampus were examined for high affinity choline uptake (HAT), choline acetyltransferase (CAT), muscarinic receptors and succinate dehydrogenase levels at 3, 20, 68, and 92 hours after injection. At 20, 68 and 98 hours after injection a statistically significant decrease in HAT was observed in hippocampus and the basal ganglia, but not in the frontal cortex. No change in HAT was observed in any brain region at the 3 hour time point. A statistically significant decrease in CAT was seen in the frontal cortex only. Muscarinic cholinergic binding was studied using $^3$(H) quinuclidinyl benzilate (QNB) as the ligand. All three areas studied showed a significant decrease of 20-30% in the binding of QNB. Other mustards such as AF64A have been shown to interact with the HAT and the CAT systems. (Mantione, C. et al. Science 213, 579, 1981). However no effect of AF64A on the cholinergic binding site was demonstrated. SHM is the first mustard to our knowledge to have an effect on both the pre- and postsynaptic functions of acetylcholine. At the concentration of 2.5 mg/kg, SHM does not have an effect on the activity of the succinate dehydrogenase. Higher concentrations, 5 mg/kg, do cause a decrease in the activity of succinate dehydrogenase of 30% in the hippocampus and the frontal cortex. The existence of a link between the change in succinate dehydrogenase activity and the alterations in the cholinergic system are being studied. Our data to date indicate that cholinergic neurotoxicity is one of the mechanisms of action of SHM. The effect of SHM on other neurotransmitter systems is currently being examined to establish its specificity. SHM could be useful as a chemotherapeutic agent in the management of malignancies of neuroectodermal origin, especially if these are demonstrated to have an active choline transport system.

317.10 DENERVATED FROG SKELETAL MUSCLE HAS ACETYLCHOLINE-ACTIVATED CHANNELS WITH UNIQUE CONDUCTANCE AND LIFETIME PROPERTIES. C.N. Allen and E.X. Albuquerque. Dept. Pharmacol. & Exp. Therap., Univ. Maryland Sch. Med., Baltimore MD 21201.

Chronic denervation of either mammalian or amphibian skeletal muscle results in a spread of acetylcholine (ACh) sensitivity to nonjunctional regions of the muscle. Results of fluctuation analysis studies demonstrate that the ACh-activated channels of the extrajunctional regions have a lower conductance than those of the junctional region. We used the patch clamp technique to evaluate the characteristics of the ACh channels of the extrajunctional region of chronically denervated skeletal muscles. The interosseal muscles of Rana pipiens were denervated, under chloral hydrate anesthesia, by removing a 1.5 cm section of the right sciatic nerve. The left side remained innervated and served as a control. The muscles were enzymatically dispersed and the single fibers mounted in a recording chamber using a parafilm-paraffin oil adhesive. Innervated muscles contained a single population of ACh-activated channels with a conductance of 28 pS at membrane potentials between -40 and -170 mV. Following chronic denervation (42-44 days), two classes of ACh-activated channels having conductances of 17 and 28 pS could be recorded from a single cell attached patch. The conductance of the larger channel is 65% greater than the low conductance channel and is therefore not the result of the simultaneous activation of two low conductance channels. Histograms of the lifetimes of the channels of innervated and denervated muscles were fit by a single exponential function at all the membrane potentials tested. Both classes of channels from the denervated muscle had lifetimes longer than those of the innervated muscle over the entire membrane potential range. The lifetimes of the innervated muscle channels showed an e-fold increase with a hyperpolarization of 70 mV. The low and high conductance channels of denervated muscle had an e-fold increase in lifetimes with membrane potential changes of -100 mV and -120 mV, respectively. These data indicate that nerve transection causes a new class of ACh-activated channels to appear which have lifetime and conductance properties different from those of innervated muscles. (Supported by USPHS Grant NS-12063 and U.S. Army Med. Res. and Develop. Command Contract DAMD-17-84-C-4219.)

317.11 BIOSYNTHESIS OF THE TORPEDO CALIFORNICA ACETYLCHOLINE RECEPTOR α-SUBUNIT IN SACCHAROMYCES CERVISIAE. N. Fujita[*1], N. Nelson[*1], T. Fox[*2], T. Claudio[3], J. Lindstrom[4], and G. Hess[1]. [1]Biochemistry, and [2]Genetics and Development, Cornell University, Ithaca, NY 14853; [3]Physiology, Yale University, New Haven, CT 06511; [4]The Salk Institute, San Diego, CA 92138.

The entire structural gene for the α-subunit of the T. californica acetylcholine receptor, isolated as a cDNA cloned fragment, was inserted into the yeast expression vector pMAc561. The resulting plasmid, pYTcα1 was used to transform the Saccharomyces cervisiae strain TD4 to the $Trp^+$ phenotype.

Total cellular proteins were separated by SDS-polyacrylamide gel electrophoresis, blotted to nitrocellulose and reacted with a mouse monoclonal antibody against the T. californica receptor α-subunit. Yeast cells that contained the plasmid pYTcα1 contained a protein that was immunoreactive and had the same mobility as the α-subunit from T. californica membranes. Yeast cells containing pMAc561 without the α-subunit and untransformed cells did not contain this protein.

Insertion of the α-subunit into the yeast plasma membrane was confirmed by immunofluorescent detection on intact spheroplasts. Spheroplasts of transformed cells containing pYTcα1 were incubated with an α-subunit-specific monoclonal antibody, washed and decorated with a second antibody conjugated to fluorescein iso-thiocyanate. To control for the possibility that antibodies might penetrate the plasma membrane, even though they were not permeabilized, spheroplasts were also treated with antibody against porin, a major protein of the outer mitochondrial membrane. In contrast to the result with the anti-α-subunit antibody, treatment with anti-porin did not result in positive immunofluorescence, unless the spheroplasts were first permeabilized by treatment with Triton-X100.

Plasma membranes containing the α-subunit reacted with [$^{125}$I]-α-bungarotoxin with a $K_d$ value of 0.2 μM. Plasma membranes which did not contain the α-subunit did not react.

The results indicate that yeast can express the α-subunit of the nicotinic acetylcholine receptor efficiently and insert it into its plasma membrane.

This work was supported by a grant from the Cornell Biotechnology Program, which is supported by the New York State Science and Technology Foundation and a consortium of industries.

317.12 AUTORADIOGRAPHIC AND ELECTROPHYSIOLOGICAL EVIDENCE FOR NICOTINE RECEPTORS ON ASCENDING FOREBRAIN DOPAMINE NEURONS. P.B.S. Clarke[1], D.W. Hommer[2], L.R. Skirboll[2], and A. Pert[1]. Biological Psychiatry[1] and Clinical Neuroscience[2] Branches, NIMH, Bethesda, MD 20205.

Although binding of the putative nicotinic receptor ligand alpha bungarotoxin is low or absent in the substantia nigra (SN) (Clarke, P.B.S., et al, J. Neurosci., in press), high affinity specific binding of $^3$H-nicotine ($^3$H-N) is densely represented in the SN pars compacta (SNC) with little labeling in the pars reticulata (SNR) (Clarke, P.B.S., et al., Brain Res., 323:390, 1984). Using autoradiography and electrophysiology, we examined the relationship between this nicotinic binding site and the nigrostriatal dopamine (DA) system.

Autoradiography: Rats (n=6) received a unilateral injection of 6-OHDA into the medial forebrain bundle and were sacrificed 5 weeks later for $^3$H-N receptor autoradiography (as in ref. 1 but using $^3$H-L-nicotine and computer-assisted densitometry). Striatal DA was assayed by HPLC. Electrophysiology: SNC and SNR were recorded in chloral hydrate anaesthetised rats. Units were distinguished by spike shape, firing rate and pattern, response to apomorphine and haloperidol (both 0.1 mg/kg iv), and by histological verification of recording site.

Unilateral 6-OHDA lesions resulted in a near-total depletion of DA in the ipsilateral striatum and a marked reduction in Nissl staining in the ipsilateral SNC. Concomitantly, $^3$H-N labeling was reduced in the ipsilateral striatum and SNC compared to the contralateral side. Labeling was also reduced in the cell body and terminal areas of the mesolimbic DA pathway, but not in the frontoparietal cortex which lacks a DA innervation.

DA neurons of the SNC were stimulated by both iv (2-128 μg/kg) and sc (1 mg/kg) L-nicotine bitartrate (dose as base). The stimulant action of sc nicotine was blocked by the centrally active antagonist mecamylamine (MEC, 2 mg/kg iv) but not by the peripheral antagonist chlorisondamine (CHL, 0.1 mg/kg iv). In contrast, although SNR neurons were markedly stimulated by iv nicotine, pretreatment with either MEC or CHL was effective in blocking this effect.

Thus, the autoradiographic distribution of $^3$H-nicotine binding sites within the SN is consistent with electrophysiological findings that the SNC is one of the sites of nicotine's central actions.

317.13 PRESYNAPTIC MODULATION OF ACETYLCHOLINE RELEASE. G.V.W. Johnson and R.S. Jope. Department of Pharmacology, University of Alabama, Birmingham, AL 35294.

The modulation of stimulated acetylcholine release is still a poorly understood process. One putative modulator that has been discussed is phospholipase $A_2$. Three inhibitors of phospholipase $A_2$, quinacrine, chloroquine and 4-bromophenacyl bromide, reduced the rate of $K^+$-stimulated acetylcholine release from rat cortical slices. The effects of quinacrine, the most potent of these inhibitors of $K^+$-stimulated acetylcholine release, was studied in greater detail using [$^2H_4$]choline as a tracer to distinguish between the release of newly synthesized and endogeneous acetylcholine. Incubation with varying concentrations ($10^{-6}$ M to $10^{-3}$ M) of quinacrine produced similar inhibition profiles for the release of both newly synthesized and endogeneous acetylcholine, except the release of newly synthesized acetylcholine was inhibited to a greater extent at the two highest concentrations of quinacrine. Incubation of cortical slices in the presence of quinacrine resulted in acetylcholine accumulation within the tissue, apparently due to the inhibition of release, and a reduction in the production of labelled acetylcholine, indicating an inhibitory effect on acetylcholine synthesis. Quinacrine also was a potent inhibitor ($IC_{50}$ = 5 x $10^{-6}$ M) of synaptosomal high affinity choline transport.

The effects of AH5183, the most potent known inhibitor of the vesicular loading of acetylcholine (Anderson et al., Molec. Pharmacol. 24:48, 1983), were also examined on these processes. AH5183 inhibited the $K^+$-stimulated release of newly synthesized acetylcholine to a greater degree than of endogeneous acetylcholine. AH5183 also induced an accumulation of endogeneous acetylcholine in the cortical slices. Synaptosomal high affinity choline transport was inhibited by AH5183 with an $IC_{50}$ of approximately 2 x $10^{-5}$ M.

These data suggest that the AH5183-induced inhibition of $K^+$-stimulated acetylcholine release is directly related to this compound's inhibition of the transport of acetylcholine into the vesicles. Quinacrine which has also been reported to be a potent inhibitor of vesicular acetylcholine loading, also effectively inhibited $K^+$-stimulated acetylcholine release. In addition, quinacrine is known to inhibit phospholipase $A_2$. It is unclear whether quinacrine and AH5183 are inhibiting acetylcholine release through similar sites. However, these results do raise the interesting possibility that phospholipase $A_2$, which has been reported to be located in synaptic vesicles (Moskowitz et al., Sci. 216:305, 1981), may play a modulatory role in the vesicular loading and release of acetylcholine.

318.1 POSTSYNAPTIC POTENTIALS IN AXOTOMIZED AND X-REGENERATED CAT MEDIAL GASTROCNEMIUS (MG) MOTONEURONS. R.C. Foehring, G.W. Sypert, and J.B. Munson. Dept. of Neuroscience, University of Florida, College of Medicine, Gainesville, Florida 32610.

We tested whether alterations in mono- or polysynaptic inputs to MG motoneurons (MNs) accompanied axotomy and/or subsequent regeneration. The medial gastrocnemius (MG) and lateral gastrocnemius-soleus (LG-S) nerves were sectioned peripherally, removing all muscle afferent input from the triceps surae. The nerves were re-sutured to the distal stump of the other triceps nerve (X-regeneration). We examined composite monosynaptic Ia EPSPs from LG-S nerve, and polysynaptic PSPs from the sural nerve, by intracellular recording from MG MNs up to 9 months post-operatively. Ia monosynaptic EPSPs were reduced in amplitude following nerve section and recovered incompletely following regeneration. There was no evidence for myotypic respecification of Ia input following X-reinnervation.

| | + | +/- | - | (N) | AP* |
|---|---|---|---|---|---|
| FAST MNS | | | | | |
| Normal | 17% | 73% | 10% | (41) | 79 |
| X-Regenerated | 4% | 86% | 10% | (50) | 82 |
| SLOW MNS | | | | | |
| Normal | 0% | 22% | 78% | (9) | 75 |
| X-Regenerated | 4% | 64% | 32% | (28) | 79 |
| ALL MNS | | | | | |
| Normal | 14% | 64% | 22% | (50) | 78 |
| X-Regenerated | 4% | 78% | 18% | (78) | 81 |
| Axotomy | 6% | 65% | 29% | (17) | 80 |

* Action Potential Amplitude

Electrical stimulation of sural afferents (3xThreshold) resulted in an early EPSP followed by an IPSP in normal fast MNs (FF+FI+FR). In a few cases the EPSP (voltage-time integral) was dominant (+; EPSP/IPSP>1); in most cases there was an EPSP/IPSP mixture (+/-; 0.05<EPSP/IPSP<1). Normal type S MN PSPs typically were dominated by the IPSP (-; EPSP/IPSP<0.05). In X-regenerated MNs an increased number of type S units had the EPSP-dominant or mixed pattern. It did not matter which muscle (LG or Soleus) was X-reinnervated. Consistent results were obtained from MG self-regenerated MNs. We feel the altered PSP pattern in type S motoneurons may relect neural plasticity within the spinal cord, probably of the last order interneurons in the early EPSP circuit. Sprouting of new connections and unmasking of existing synapses are both consistent with these data. Supported by NIH grant NS15913, the MRS and RERDS of the VA, and the W.L. Gore Co.

318.2 EXERCISE INDUCED REMODELLING OF FAST & SLOW TWITCH NEUROMUSCULAR JUNCTIONS IN RAT. M.H. ANDONIAN AND M.A. FAHIM. Andrus Gerontology Center, University of Southern California, L.A., CA 90089-0191.

The effect of endurance exercise on morphometric parameters of rat neuromuscular junctions (NMJ) was examined in two functionally distinct muscles, the tonic soleus (SOL) and the phasic extensor digitorum longus (EDL). Sprague-Dawley rats (180-220 grams) were trained on a rodent treadmill for 30 min./day, 30 m/min (≅ 70% $VO_2$ max) up a 10% grade for 30 consecutive days without electrical stimulation. Under Metofane anesthesia, EDL and SOL muscles were dissected from these animals and three age-matched controls stained with the zinc-iodide osmium technique. Camera lucida drawings were made 20-30 endplates (>90% visible) from each muscle. Morphometric measurements, including the perimeter of the endplate, area, extent length and fiber diameters were made by digitizing the drawings on a computer controlled bit pad.

The NMJs of the control SOL and EDL were relatively simple and unbranched in appearance. Those of the exercised animals were larger, and more complicated. Fiber diameters of the exercised SOL and EDL were 15%-25% larger than controls. Fiber diameter, endplate perimeter, area and extent lengths of the exercised SOL and EDL muscles were normalized for changes in fiber diameter found to be signficantly ($P < .001$) greater than controls. Exercised SOL and EDL endplates had significantly ($P < .05$) more branches and sprouts than the controls.

These data suggest that the increased neural stimuli muscles receive during training results in remodelling at the NMJ. The less actively recruited EDL shows greater changes than the continually active SOL which suggests that basal muscular activity is also a factor in determining the structure of the NMJ.

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318.3 TRAINING EFFECTS ON THE RECOVERY OF FULL WEIGHT-BEARING STEPPING IN ADULT SPINAL CATS. R.G. Lovely, R.J. Gregor, R.R. Roy, and V.R. Edgerton. Brain Research Institute and Department of Kinesiology, UCLA, CA 90024.

Some cats cordotomized when adults can recover full weight-bearing stepping (Neurosc. Abst. 47.1, 1982; Neurosc. Abst. 184.1, 1984). The time course for their recovery and the effects of training have not been examined. This study was designed to monitor changes in the locomotor capabilities of adult spinal cats trained over a 5 month period and to compare their performance with an untrained cordotomized group.

Transection of the spinal cord at T12-T13 was performed on 16 adult mongrel cats. One month post-transection, 14 cats were capable of full weight-bearing (FWB) stepping on a motor driven treadmill if the tail was pinched or crimped. Within the treadmill apparatus, the animals were stabilized by a harness about the shoulder girdle and the forepaws were on a platform 1" above the treadmill belt. Top speeds for FWB were 0.05-0.2 m/s at this time. Of the 14 FWB cats, 8 were selected at random for 30 minutes of treadmill exercise per day, 5 days/week (TE group). The rest were assigned to a transected non-exercised group (TNE). Daily records were kept on treadmill speeds, time at each speed, step rate at each speed, and the number of steps that were not FWB. The number of FWB steps times treadmill speed was used as a measure of performance.

The TE cats reached a performance plateau 25-75 days after initiating the exercise regimen. Maximum FWB speeds were 0.4-0.85 m/s. Only 2 TE cats required tail pinching to take more than 30 consecutive steps after 5 months of training. On some days, 5 of the 8 TE cats could locomote for the entire session without trainer assistance of any kind. In TNE cats, maximum FWB speeds were 0.15-0.35 m/s 6 months post-transection and no more than 5-7 consecutive steps could be taken without tail pinching.

The performance of 3 TE cats declined after initial improvement. Declining performance was associated with increasing frequency of landing on the dorsum of the paw at paw contact, ankle hyperextension, and insufficient extensor activation to support body weight.

Although some recovery occurred without training, TE cats performed better than TNE cats 6 months post-transection. While training may have affected the neurological apparatus controlling stepping, it may also have facilitated performance by retarding the progression of muscle abnormalities (Roy et al., accompanying abstract) and joint abnormalities that can occur as a result of transection. Supported by NIH grant NS 16333.

318.4 LONG-TERM EXERCISE EFFECTS ON THE MECHANICAL PROPERTIES OF THE SOLEUS MUSCLE IN ADULT SPINAL CATS. R.R. Roy, R.J. Gregor, R.G. Lovely, K.M. Baldwin, and V.R. Edgerton. Brain Research Institute and Kinesiology Dept., UCLA, CA 90024 and Physiology Dept., UCI, Irvine , CA 92717.

This study was designed to determine the effects of daily exercise on the skeletal muscle below the level of the lesion in low thoracic (T12-T13) transected (T) adult cats. One group of transected cats was trained (TE) on a treadmill 30 min/day, 5 days/week such that the cats had to fully support their body weight when exercising (Lovely et al, accompanying abstract). Another group of transected cats were non-exercised (TNE). The soleus (SOL) was chosen for study because it is severely affected by spinal transection (Roy et al., JAP 56:1594, 1984) and it is heavily recruited during treadmill exercise in T cats as indicated by EMG and tendon force records (unpublished observations). Six months after transection, the *in situ* isometric contractile properties of TNE and TE SOL muscles were determined at 36±1°C and compared with data from sedentary, control (C) adult cats. SOL wet weight (MW) and maximum tetanic tension (Po) decreased by 36 and 41 %, respectively, in the TNE group, whereas the TE cats showed only a 14 and 18 % decline in the same parameters. The maximum twitch tension (Pt) showed a similar pattern. The SOL in the TNE and TE groups had shorter contraction times (CT) and one-half relaxation times (1/2 RT) than did control cats. Although the relative amount of maximum tension produced at 20 Hz (%Po) was decreased in both groups, the change in TNE cats was 15 % larger than in the TE group.

Thus, our preliminary results suggest that in the SOL there is an ameliorating effect of daily exercise involving full weight support on the atrophy and the "conversion" towards a faster muscle associated with spinal transection in adult cats.

| | n | MW (g) | Pt (g) | Po (kg) | CT (ms) | 1/2RT (ms) | P20 (%Po) |
|---|---|---|---|---|---|---|---|
| C | 10 | 3.6 | 590 | 2.2 | 98 | 99 | 91 |
| | | ± 0.3 | 39 | 0.2 | 1 | 1 | 1 |
| TNE | 4 | 2.3 | 332 | 1.3 | 54 | 51 | 75 |
| | | ± 0.4 | 40 | 0.3 | 6 | 7 | 3 |
| TE | 4 | 3.1 | 540 | 1.8 | 62 | 48 | 86 |
| | | ± 0.6 | 46 | 0.2 | 4 | 3 | 1 |

Mean±S.E.

Supported by NIH Grant NS 16333

318.5 THE EFFECTS OF HYPERTHYROIDISM UPON SLOW OXIDATIVE AND FAST GLYCOLYTIC MOTONEURON ENERGY METABOLISM. D.W. Sickles, *T.G. Oblak* and J. Scholer.* (SPON: G. Standage) Department of Anatomy, Medical College of Georgia, Augusta, GA 30912

Motoneurons innervating different types of muscle fibers are specialized with respect to quantitative differences in the activity of enzymes of oxidative metabolism (Sickles and Oblak, J Neurophysiol. 51;529, 1984). Despite extensive efforts to elucidate regulatory mechanisms of muscle fiber differentiation, similar studies in motoneurons have not been considered. The thyroid status of the animal influences physiological and histochemical properties of the muscle; hyperthyroidism increases speed of contraction and converts slow oxidative (SO) fibers to fast oxidative glycolytic (FOG) (Fitts et al,. Am J Physiol 238: C15, 1980). This study was undertaken to determine whether motoneuron metabolism is thyroid hormone regulated. Eight 170 g male Sprague-Dawley rats were fed T3 (1mg/kg food) and T4 (3mg/kg food) in their diet for 6-7 weeks, 6 controls were given only the powdered chow. The latter gained an average of 91% of starting weight, experimentals only 64.7%. At the termination of the experiment plasma T4 levels were elevated 57.8% and heart/body weight ratios were 54.7% higher in experimentals. Fiber populations of the soleus changed from 86.9% SO, 10.1% FOG, 2.8% Intermediate to 20.4% SO, 43.9% FOG and 34% Int. No significant change in tensor fascia lata (94% fast glycolytic fibers) fiber populations were observed. The NADH-TR (oxidative metabolism) and α-glycerophosphate dehydrogenase (glycolytic) activities of the soleus were changed by +46.2% and +357.1%, respectively. NADH-TR activity of TFL muscle fibers was slightly increased (5.4%). A 16.6% increase in soleus (SO) motoneuron NADH-TR activity was observed in hyperthyroid rats; only a 4.9% change in TFL motoneuron NADH-TR activity was found- (see Sickles and Oblak, J Neurophysiol 51: 529, 1984 for method details). We conclude that thyroid hormone causes an increase in the oxidative metabolism of motoneurons, indicating a direct thyroid CNS effect. We have verified the quantitatively greater effect of thyroid hormones upon SO muscle fibers and, in addition, we have shown a differential effect of the hormones on motoneuron oxidative metabolism.

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318.6 EFFECT OF SYNERGIST TENTOMY ON THE OXIDATIVE METABOLISM OF RAT SOLEUS MUSCLE AND MOTONEURONS. J.K. Pearson*, D.W. Sickles* (SPON: M. Mulroy), Department of Anatomy, Medical College of Georgia, Augusta, GA 30912

Tenotomy of synergists (gastrocnemius and plantaris) to the soleus muscle of the rat results in conversion of muscle fiber type populations from 84% slow oxidative (SO) and 16% fast oxidative glycolytic (FOG) to a pure population of SO fibers within 60 days after tenotomy (Iannuzo et. al., Life Sciences 19: 1517, 1976). This system has been used as a model of "functional overload" which suggests increases in tension and/or overall activity. We have assessed the changes occuring under these conditions in the soleus muscle using histochemical methods for ATPase (Guth and Samaha, Exp. Neuro. 28: 365, 1970), NADH-tetrazolium reductase (NADH-TR; oxidative metabolism) (Scarpelli et. al., J. Biophys. Biochem., Cytoche. 4: 747, 1958), and menadione-linked α-glycerophosphate dehydrogenase (α-GPDH; glycolytic metabolism) (Wattenberg and Leong, J. Histochem. Cytochem. 8: 296, 1960). Motoneuron metabolism varies in accordance with the type of muscle fibers innervated (Sickles and Oblak, J. Neurophys., 51: 529, 1984). The effects of functional overload on motoneuron metabolism has never been examined. We determined the changes in motoneuron NADH-TR activity and correlated these with changes in muscle fiber type population. Sprague-Dawley rats (150-174g) underwent tenotomy of the left plantaris and gastrocnemius (including removal of 1/2 of the muscles) under pentobarbital anaesthesia. Sham operated and unoperated animals served as controls. Sixty days later, soleus motoneurons were identified via intramuscular injections of HRP (Sigma Type IV) (Sickles and Oblak, J. Neurophys 51: 529, 1984). No significant differences in muscle or motoneuron enzyme activities were observed between sham and unoperated animals. The ATPase staining of the experimental muscles did show a conversion from FOG to SO fibers. The NADH-TR activity of the soleus decreased 13.7% from control, while the NADH-TR activity of the motoneurons showed a parallel decrease of 16.3%. The α-GPDH activity in the experimental soleus was decreased from control. We have shown that tenotomy of synergists to the soleus does not increase the oxidative metabolism of the soleus muscle and conclude this is not a model for increased activity. The change in the myosin ATPase does suggest that tension rather than activity may control the expression of myosin type. The decrease in the oxidative metabolism of the motoneuron paralleled the decrease in NADH-TR activity of the muscle fibers demonstrating a strong relationship of this parameter within the motor unit.

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318.7 EVIDENCE FOR THE CONTINUING ROLE OF TARGETS IN CONTROLLING MOTONEURON TERMINALS IN ADULT MAMMALS. R. Butler. Department of Anatomy, McMaster University, Hamilton, Ontario, L8N 3Z5.

A review of the evidence regarding interneuronal synaptogenesis indicates that the transformation of growing axons into specialized nerve terminals is clearly regulated by their synaptic targets. This is especially true for vertebrate neuromuscular junctions where peripheral motor axon terminal differentiation seems to be under local control by certain post synaptic membrane specializations, especially the basal lamina. There are also suggestions that these dynamic relationships are maintained with increasing age in normal adult muscle and that neuromuscular junctions can undergo continual remodelling. I have undertaken two sets of experiments which support the concept that mature targets can modify alpha motoneuron morphology. The first deals with the reinnervation of intact targets by regenerating alphas. Following crush injuries to the nerve to the tenuissimus muscle in adult cats, single regenerating alphas were found with different intrafusal effects in different muscle spindles, implying that different branches of the same alpha were functionally innervating different types of intrafusal muscle fibre simultaneously. Reconstructions showed that different branches of these single alphas also had morphologically different types of endings (focal plates and engrappe), simultaneously, on different types of intrafusal muscle fibre. The converse series of experiments deals with the influence of regenerating targets on intact motoneuron terminals. In order to make available a very different target for alpha motoneurons with focal endplates, an extraocular muscle containing multiply innervated slow tonic fibres was excised from a rat and transplanted into its own hindlimb, using the fast twitch extensor digitorum longus as host. The transplant and host were then treated with bupivacaine. The muscle degenerates and subsequently regenerates allowing the intact neurons and their terminals the option of innervating very different targets. My observations indicate that not only can intact alphas innervate the regenerated transplant but also their existing focal endplates are modified to a multiple innervation on regenerated extraocular fibres. These results support the suggestion that neuromuscular targets maintain a continuing dynamic influence on motoneurons in the adult. Supported by the Ontario March of Dimes and the Muscular Dystrophy Association of Canada.

318.8 SPECIFIC CHANGES IN TRANSPORTED RETINAL PROTEINS DURING COMPRESSION OF THE RETINOTECTAL PROJECTION IN GOLDFISH. Myong G. Yoon and Frank A. Baker*; Department of Psychology, Dalhousie University, Halifax, Nova Scotia, Canada. B3H 4J1.

In addition to the ability of regeneration of optic fibers after axotomy, an adult goldfish is also capable of reorganizing the topographic pattern of the visual projection from the retina to the mid-brain visual center, the optic tectum. If the caudal half of the tectum is ablated, the remaining rostral hemitectum accommodates optic fibers not only from the proper temporal half of the retina but also from the previously inappropriate nasal half in an orderly compressed topographic pattern. This field compression suggests that extensive reorganization of synaptic contacts may occur in the halved tectum. To test whether retinal ganglion cells undergo specific changes in their metabolism and axonal transport of proteins during the field compression the caudal half of the left tectal lobe was excised after bilateral optic nerve crush in adult goldfish. At various intervals between one and four months after surgery, retinal proteins transported in the right optic nerve (regenerating to the halved left tectal lobe) were labelled with $C^{14}$-proline and those in the left optic nerve (regenerating to the whole right tectal lobe) with $H^3$-proline. For each time-point, both optic nerves were mixed together and co-processed for SDS gel electrophoretic separation of the differentially labelled proteins. The relative labeling spectra of various proteins were compared between the two isotopes for each time-point. A group of rapidly-transported proteins with molecular weights between 180 and 210 kilodaltons (Kd) increased differentially in the right optic nerve ($C^{14}$) compared with the left optic nerve ($H^3$) during the 45-55 days post-operative period. This specific change suggests that the 180-210 Kd proteins may be involved in the retinotectal field compression, which occurs at about the same period in adult goldfish.

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318.9 PLASTICITY OF SYNAPTIC VESICLE NUMBER IN ADULT RAT SUPERIOR CERVICAL GANGLION FOLLOWING DEAFFERENTATION AND GANGLIONIC BLOCKADE. K.F. Greif Dept. of Biology, Bryn Mawr College, Bryn Mawr, PA 19010.

Changes in the number of synaptic vesicles in the adult rat superior ganglion (SCG) following deafferentation or pharmacological blockade of synaptic activity have been monitored using a monoclonal antibody directed against a 65 kD integral membrane protein associated with synaptic vesicles (SV) (Matthew et al, J. cell biol. 91:257, 1981). Previous studies have shown that levels of this antigen undergo significant postnatal changes during normal development and after neonatal deafferentation.

SCGs in adult rats were deafferented by bilateral section of the cervical sympathetic trunk. Levels of antigen were measured by radioimmunoassay at 7 or 30 days following surgery. For studies of synaptic activity, rats were injected twice daily for 7 days with chlorisondamine (10 mg./kg, s.c.) and then sacrificed immediately.

Seven days following bilateral deafferentation, levels of SV were significantly increased to 46% above control levels. No decrease in SV levels was observed in iris, a primary SCG target tissue, suggesting that the increase in SV in the SCG is not the result of blocked axonal transport. Immunohistochemical staining of frozen sections of SCG confirmed the loss of labelling in presynaptic terminals, indicating that increased amounts of SV accumulate in ganglion cell perikarya. By 30 days after surgery, SV levels decrease to 30% of control levels, a value similar to that observed 30 days after neonatal deafferentation. After 7 d. treatment with chlorisondamine, enhanced increase in SV levels over that in saline-treated animals is observed, consistent with a real increase in antigen levels in the presence of intact presynaptic terminals.

These results suggest that activity of ganglionic neurons contributes to the regulation of synaptic vesicle numbers in the adult rat SCG and that significant plasticity in vesicle numbers can occur. However, the time course of response differs from that observed in neonatal animals. These findings are of interest in view of reported changes in plasticity of phenotypic expression of neurotransmitters in the SCG in adult and aged rats.

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318.10 STIMULATION OF PLASTICITY CHANGES WITHIN THE LESIONED ADULT RAT RETINA. J. E. Turner. Department of Anatomy, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27103.

We report for the first time that by employing a new lesion paradigm in the adult rat retina there is evidence for plasticity changes involving a dramatic increase in the cellularity of the inner nuclear layer which may be due to a transformation of photoreceptor cells. A small incision perpendicular to the long axis of the optic nerve is made through the sclera, choroid and retina on the dorsal surface of the eyes of young adult male Sprague-Dawley rats. The retina is gently detached at the lateral edges of the incision with a small glass stylus and the wound closed with microsutures and 10-0 thread. Experimental lesion groups received a desheathed peripheral nerve implant into the lesion site while controls had none. Eyes were analyzed for light and electron microscopic morphometric observations at 1, 2, 4, 6 and 8 weeks after lesion. The retinal detachment process causes an irreversible degeneration of the photoreceptor cell (PRC) outer segments followed by PRC cell survival in controls for at least 6 to 8 weeks after lesion. In experimental lesion sites there was a rapid disappearance of PRCs which was correlated with a dramatic increase in the cellularity of the underlying inner nuclear layer (INL). The increase in INL cells was not due to increased Muller cells, retinal pigment epithelium or macrophages. Cells in the diminishing outer nuclear layer (ONL) were seen in transitional states between normal PRC and INL neuron morphologies. In addition, the sum of cell counts in the intact control ONL added to these in the underlying intact INL were not significantly different from counts of cells in the expanded INL of comparable experimental retinas where the ONL had completely diminished. In experimental retinas an increase in INL thickness was also accompanied by a greatly thickened underlying inner plexiform layer. The transformation process appears to be initiated and/or greatly accelerated by the presence of a peripheral nerve implant. Thus the retina is capable of being induced to initiate rather profound cellular changes. Supported by Grant EY 04377 from the National Eye Institute awarded to J.E.T.

318.11 PARTIAL DEAFFERENTATION INDUCES INCREASED DOPAMINE RECEPTOR DENSITY AND DOPAMINE-SENSITIVE ADENYLATE CYCLASE, WHILE DECREASING ($Na^+$-$K^+$)-ATPase AND OUABAIN BINDING IN THE OLFACTORY TUBERCLE. R.B.LINGHAM*, A.C.SWANN, V.HOLCOMBE*, S.A.KHAN*, G.A.ROBISON, AND Z.GOTTESFELD. Depts. Pharmacol. Psychiatry, and Neurobiology & Anatomy, Univ. Tex. Med. Sch., Houston, TX 77025.

Destruction of a major non-catecholaminergic input from the olfactory bulb (OB) elicits collateral sprouting of dopaminergic (DA) axons in the olfactory tubercle (OT). This phenomenon has been identified by the increased density of axon terminals containing tyrosine hydroxylase (TH) and by enhanced uptake of DA into synaptosomes of the OT (Gilad, G.M. and Reis, D.J., Brain Res. 160:17, 1979). The functional consequences of this innervation are presently not known. This study examined the effect of lesioning the OB on 1) density of DA receptors, 2) the DA-sensitive adenylate cyclase (AC) and 3) ($Na^+$-$K^+$)-ATPase activity as well as 4) levels of $^3H$-ouabain binding in OT membranes.

Rats (200-300 g) were lesioned or sham-operated under chloropent anesthesia. Animals were killed at specific time intervals post-operative and the OT were removed and processed for further chemical analyses. Membranes were prepared by standard techniques of homogenization and differential centrifugation, then assayed for AC (Salomon, Y. Adv. Cyc. Nucl. Res. 10:35, 1979) and DA receptor binding (Reisine et al., Life Sci. 21:335, 1977). ($Na^+$-$K^+$)-ATPase activity, in homogenates or microsomes, and $^3H$-ouabain binding were measured according to Swann and Albers (Biochem. Biophys. Acta, 382: 437, 1975) and Swann (J. Pharmacol. Exp. Ther., 228:304, 1984), respectively.

The results indicate that DA-, NaF- and forskolin-stimulated AC activity increased as early as 7 days and reached a maximum (25%) by 20 days post-lesioning. DA-receptor binding increased (25%) during this time period and correlated with the increase of TH activity in the sprouting DA fibers (Gilad and Reis, ibid). Higher levels of GTP- and NaF- stimulated AC activities were found in detergent extracts of OT membranes from 20-day lesioned rats. ($Na^+$-$K^+$)-ATPase activity and ouabain binding decreased 30% and 25%, respectively, 20 days after lesioning.

In summary, lesion-induced DA sprouting in the OT is associated with 1) increased AC activity, 2) a comparable increase in DA-receptor binding, 3) increased levels and/or activity of the stimulatory GTP-binding protein associated with AC and 4) decreased ($Na^+$-$K^+$)-ATPase activity and specific ouabain binding.

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318.12 LESION-INDUCED TRANSNEURONAL CELL DEATH AND DEGENERATION S.F. Hoff, Department of Pharmacology, The Chicago Medical School, North Chicago, IL 60064.

After unilateral ablation of the entorhinal cortex, we have reported both degenerative and non-degenerative changes in synaptic density throughout the entire ipsilateral and contralateral molecular layer of the hippocampal dentate gyrus in adult rats. The ipsilateral dentate granule cells are partially denervated by the lesion (85% loss of input). In addition we have observed a complete cycle of synapse turnover within the ipsilateral (but not contralateral) CA4/Hilus region of the hippocampal formation, along with a significant bilateral increase in the density of the mossy fiber terminals (MFT) arising from the granule cell axons. The purpose of the present study was to determine if a degenerative process was involved in the bilateral transneuronal response to a distant injury.

Male Sprague-Dawley rats (90 days old) received a complete unilateral electrolytic lesion of the entorhinal cortex. The animals were allowed to survive for 4, 10, 60 or 180 days post-lesion and were then sacrificed and prepared for electron microscopy. Over the experimental time course, EM montages were taken across the CA4/Hilus region along a path joining the midpoints of the dorsal and ventral blades of the dentate granule cell layers. Approximately 161,000 square microns of neuropil have been evaluated for this study.

Within the ipsilateral CA4/Hilus region we have observed a 40% decrease in synaptic density at 4 days post-lesion, which was followed by a return to normal density by 180 days post-lesion. Also, there was a 2.25 fold increase in MFT density during the experimental time course. During this study, twenty-three synaptic profiles were classified as degenerating (15 dendritic elements and 8 axonal terminals). These profiles represented about 0.12% (23/19561) of the total synaptic profiles counted in the ipsilateral side. Also, two degenerating cell bodies were observed in the CA4/Hilus area at four days post-lesion.

Within the contralateral CA4/Hilus area, we have observed no change in the normal synaptic density over the time course. However, there was a 2.5 fold increase in the density of MFT. In this area only six degenerating profiles were observed and these accounted for 0.05% (6/12833) of the total synaptic profiles. No degenerating cell bodies were seen.

The small amounts of degeneration reported in this study do not account for the initial 40% loss in ipsilateral synaptic density or the pronounced increase in density of the MFT. Along with an apparent non-degenerative mechanism of synapse turnover, the degenerative process may represent a simple "dying back" of some synaptic contacts during rearrangement of the circuitry interactions of the dentate granule cells and CA4/Hilus neurons.

318.13 HIPPOCAMPAL SYMPATHETIC INGROWTH IN RATS AND GUINEA PIGS: QUANTITATIVE MORPHOMETRY OF TOPOGRAPHICAL DIFFERENCES. J.N. Davis and R.M. Booze, VA Med. Center and Depts. of Med. (Neurology) and Pharmacology , Duke University Medical Center, Durham, NC 27705.

Sympathetic ingrowth is an unusual neural rearrangement after brain injury in which peripheral noradrenergic nerve fibers grow into the hippocampus in response to damage of the septo-hippocampal pathway. We quantified histofluorescent catecholaminergic fibers in the hippocampus of rats and guinea pigs to study the regulation of the ingrowth response.

Unilateral fornix or medial septal lesions were placed and the animals sacrificed two to six months after the lesions. Every tenth cryostat section through the hippocampus was processed for catecholamine histofluorescence (de la Torre and Surgeon, 1976). Adjacent sections were collected for cresyl violet staining (to aid in matching sections) and acetylcholinesterase staining (to verify cholinergic depletion).

Sympathetic ingrowth was found to occur in both species as a specific response to injury of the septo-hippocampal pathway. As for rats, ingrowth in guinea pigs was found to be dependent on cholinergic depletion and originating from the superior cervical ganglion. However, a species difference was observed in the topographical distribution of sympathetic ingrowth. In rats, noradrenergic ingrowth fibers were found above and below the granule cell layer, the dentate hilus, and the pyramidal cell layer $CA_3$. Ingrowth in guinea pigs also occurred in the dentate gyrus and area $CA_3$ but, unlike the rat, was prominent throughout area $CA_1$. Quantitative estimates of fiber numbers confirmed these observations and further found significant species differences in the intrahippocampal lamellar distribution of ingrowth fibers. Within area $CA_3$ of rats, the stratum oriens, stratum pyramidale, and stratum radiatum, were all found to contain similar numbers of fibers. In contrast, the guinea pig hippocampus had the greatest number of fibers localized to stratum pyramidale in areas $CA_1$ and $CA_3$. These species differences in fiber topography were not associated with any known anatomical characteristics of the hippocampus of the two species, i.e. we found no differences in the topography of mossy fibers (Timm's stain), cholinergic septal afferents (anterograde HRP), or in GAD-immunoreactive neurons.

The use of quantitative morphometric techniques has identified significant species differences in the topographical distribution of noradrenergic sympathetic ingrowth fibers in the hippocampus. These differences are challenging to current theories concerning tropic factor regulation of hippocampal ingrowth.

(Supported by the VA and NS06233)

318.14 PLASTICITY IN NEUROTRANSMITTER SYSTEMS OF THE SPINAL CORD FOLLOWING SCIATIC NERVE CRUSH.

C. Somps*, M.W. Luttges and L.J. Fisher, Aerospace Engineering Sciences, University of Colorado, Boulder, Colorado 80309.

Time-dependent alterations in spinal cord function have been observed following damage to peripheral nerves. Such changes include somatotopic reorganization and altered depth profiles for primary afferent depolarization recorded within the dorsal horn. Altered electrical responses from cross-cord and ascending pathways have been observed as well. The mechanisms responsible for these changes are unknown. However, the localized nature of the changes and the relative constancy of spinal cord morphology and protein synthesis, suggest that neurotransmitter systems may be involved in the observed plasticity. To explore this possibility, the uptake and compartmentalization of spinal cord neurotransmitters (GABA, glutamate, acetylcholine) were examined in regard to specific localizations in both dorsal and ventral horn regions. Neurotransmitter systems were examined 3, 6, & 9 days following unilateral sciatic nerve crush in mice. On each post-crush day, spinal cord tissues were removed, minced, and incubated in GBSS containing radiolabeled transmitter (GABA, glutamate) and transmitter precursor (choline chloride). After washing and homogenization, tissue samples were subfractionated using differential and density gradient centrifugations. To assure methodological constancy, double labeling was used to permit simultaneous processing of tissues from different experimental preparations. The specific activity of radioactivity recovered from synaptosome enriched subfractions was determined using corresponding Lowry protein measures based upon bovine serum albumin standards. Recovered radioactivity showed characteristic variations with time post-crush. Specific activities of recovered radioactivity were 20% below controls at 3 & 6 days post-crush for synaptosome fractions obtained from GABA, glutamate, and choline chloride labeled tissues. At 9 days post-crush, specific activities returned to near control levels for both choline chloride and glutamate. However, GABA levels at 9 days exceeded control values by more than 40%. The time course and direction of these changes are correlated with previously observed electrophysiologic differences in 6 as opposed to 9 day post-crush tests. The localization of changes to the lateralization and the level of the damaged afferents is described. That these changes corroborate neurophysiological changes indicates a short time-course (days) set of alterations in neurotransmitter systems that may underlie damage-induced changes in spinal mechanisms.

318.15 ABSENCE OF ANATOMICAL CHANGE IN THE THALAMOCORTICAL PROJECTION OF RACCOONS DURING THE PERIOD OF PHYSIOLOGICAL REORGANIZATION. D.D. Rasmusson & D.M. Nance, Depts. of Physiology & Biophysics and Anatomy, Dalhousie University, Halifax, N.S. Canada B3H 4H7

Physiological changes have been demonstrated at the level of the primary somatosensory cortex (SI) after digit amputation in raccoons and monkeys. One possible mechanism for this is an anatomical alteration in the thalamocortical projection, perhaps via sprouting.This possibility was studied by injecting one fluorescent dye into the reorganized 5th digit area and another dye into the normal 4th digit area of SI and examining the location of labelled cells in the ventrobasal thalamic area (VB). We have previously found that in the intact raccoon, the VB populations for these two digits are distinct with virtually no cells projecting to both cortical regions (Physiol. Can., 1984, 15, 173).

The present experiment was conducted in six raccoons four months following amputation of the 5th digit. The injection sites were made on the basis of the sulcus patterns and in five animals these sites were verified by electrophysiological recordings prior to injection. The dyes used were fast blue (FB) and nuclear yellow (NY) in four animals and FB and diamidino yellow (DY) in two animals. Crystals of NY and FB were used in one case, while 0.2 ul injections were used in the other animals (FB and NY, 5% solutions; DY, 1%).

The specific hypothesis tested was that cells in 4th digit VB would project into reorganized 5th digit cortex and would be seen either as double-labelled cells or as an intermingling of cells with each label. In 4 cases the results clearly contradicted this hypothesis: almost no double-labelled cells were seen and the populations were separate as in the normal animal. In the other two cases, many double-labelled cells were seen but the injection sites were found to be overlapping, due to diffusion of NY. The location of double-labelled cells within VB in these two animals was consistent with the topography of the overlap zone and was not consistent with the receptive field locations seen physiologically. Thus, these experiments suggest that anatomical changes in the thalamocortical pathway are not responsible for the observed physiological reorganization and that changes at another level of the somatosensory pathway must be responsible.

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318.16 EFFECTS OF CROSS-ANASTOMOSIS OF DIGITAL NERVES ON PRIMARY SOMATOSENSORY CORTEX OF THE RACCOON. B.G. Turnbull and D.D. Rasmusson, Dept. of Physiology and Biophysics, Dalhousie University, Halifax, N.S. B3H 4H7

Multi-unit recordings were made from the primary somatosensory cortex (SI) of four chloralose-anesthetized raccoons, 3-4 months after cutting and cross-repairing the ventral nerves of the ulnar side of the 4th digit and the radial side of the 5th. Thus, the regenerating 4th digit nerve fibres would reinnervate the glabrous skin of the 5th digit and vice versa. If the cortex simply mirrors the new peripheral innervation then each digital area of SI should be divided in half; e.g. that half of the 4th digit area which had represented the ulnar side of the digit should now respond only to the radial 5th digit, while the radial 4th representation should be unchanged. The extent to which the experimental evidence did not bear this out would indicate that central mechanisms were involved in altering the interchanged peripheral inputs.

The results showed that the affected cortical areas responded to stimulation of the reinnervated digits, but also reflected additional changes. Input from the crossed nerves was not topographically organized: map inversions and discontinuities were evident. These distortions could be explained by random regeneration of fibres across the nerve repairs. In addition to these expected inputs, the affected areas of SI also retained some responsiveness to stimulation of the original digit. For example, units in the ulnar 4th region of SI had receptive fields on both the 4th and 5th digits. These results cannot be explained by peripheral overlap from the other, intact digital nerves: the location of receptive fields and the overall topography within the affected cortex was inconsistent with this explanation and, furthermore, many of these responses remained after sectioning the other nerves during the recording session. These results suggest that the somatotopic map in SI cortex reflects central influences in addition to the altered peripheral input.

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319.1 EXPRESSION OF MHC CLASS I MOLECULES IN NORMAL BRAIN, AREA POSTREMA, AND OLFACTORY EPITHELIUM. J.P. Whelan* and L.A. Lampson. Department of Anatomy, Univ. of Penn., School of Medicine, Phila., PA 19104.

Class I molecules, which include HLA-A,B,C in man and H-2 K,D,L in mouse, are known to play an important role in the immune response. Effector phase cytotoxic T cell-recognition of foreign antigens is often dependent upon the co-recognition of appropriate class I on the target cell. Although classically described as ubiquitous, it is becoming increasingly apparent that the distribution of class I molecules varies among cell types. Neural tissues, in particular, display a paucity of class I molecules when compared to lymphoid controls (Lampson, et al. J. Immunol. 130:2471, 1983). Recently, it has been shown that the weak expression of HLA-A,B,C on neuroblastoma cell lines can be increased when the cells are treated with $\gamma$-interferon (Lampson and Fisher, PNAS 81: 6476,1984). In order to determine whether a similar modulation of class I occurs under physiological conditions in vivo, we have extended our studies to a mouse model.

An antiserum that reacts with $\beta_2$-microglobulin ($\beta_2$-m), the invariant chain associated with all class I molecules, was used in an avidin-biotin immunoperoxidase assay on both frozen and formaldehyde-fixed paraffin sections. (1) In normal mouse brain, strong stain was found in blood vessel and choroid plexus endothelium but not in the cell bodies of neurons or glia, or in myelin. This result in tissue that had been perfused with fixative confirms our earlier work in human neural tissues. (2) The area postrema, which lacks a blood/brain barrier, failed to exhibit any positive stain. Combined with the lack of positive staining in other areas of the CNS, these data suggest that weak class I expression is not only a property of neural tissue in immunologically privileged sites. (3) The olfactory mucosa, which contains an actively proliferating neural epithelium, failed to give positive staining, whereas the non-sensory respiratory epithelium did show positive stain for $\beta_2$-m. Since all layers in olfactory epithelium fail to stain, including the germinal neuroblast layer, it appears that weak class I expression may be a feature of developing, as well as post-mitotic neuronal cells. Still to be determined is whether class I modulation occurs on neurons during disease or trauma, and such work is in progress.

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319.2 REGIONAL DISTRIBUTION OF THY 1.1 IN RAT BRAIN. (SPON: J. Trubatch). C.B. Pert, J.M. Hill and R.J. Weber*. Neuroimmunology Unit, Section on Brain Biochemistry, Clinical Neuroscience Branch, National Institute of Mental Health, Building 10, Room 3N256, 9000 Rockville Pike, Bethesda, Maryland 20205-1000.

Thy-1 is a transmembrane glycoprotein of molecular weight 17,500 present predominantly on the surface of thymocytes and neurons (Williams, A.F., et al., Cold Spring Harbor Symp. Quant. Biol. 41: 51-61, 1977). Although function of Thy-1 is unclear a cell-cell adhesion role has been proposed. Because of its presence on thymocytes and neurons, the molecule constitutes a structural link between the immune and central nervous systems.

To examine the localization of Thy-1 in rat brain, we have incubated frozen sections of rat brain with a mouse monoclonal antibody to rat Thy 1.1, MRC OX-7, followed by an iodinated goat anti-mouse immunoglobulin. The sections are then exposed to X-ray film. Thy 1.1 was found to be unevenly distributed in rat brain with distinct regional differences related to laminar patterns within brain regions. This was especially evident in the cerebellum, where the granule cell layer was densely labeled, and in the hippocampal formation, where dense binding occurred in the pyramidal cell layer.

Within the hypothalamus the superchiasmatic and supraoptic nuclei were labeled. Labeling also occurred in the substantia nigra, pars reticulata, interpeduncular nucleus and central grey of the midbrain region.

Most brain regions with abundant Thy 1.1 labeling were neuron-rich areas. This observation is consistent with the previously reported findings that Thy 1.1 is found primarily on neurons.

319.3 THE DEVELOPMENTAL APPEARANCE OF THY-1 ANTIGEN IN THE AVIAN NERVOUS SYSTEM. P.L. Jeffrey, A.M. Sheppard*, and C.M. Sinclair*. Children's Medical Research Foundation, University of Sydney, N.S.W., Australia and Biochemistry Dept., Monash University, Clayton, Vic., Australia.

A monoclonal antibody against chicken Thy-1 glycoprotein (BSJ-1) was utilized to investigate the developmental appearance of Thy-1 in the chicken nervous system by (a) an immunohistochemical technique using a modified peroxidase antiperoxidase technique and (b) quantitative distribution by an ELISA technique and $^{125}$I-Fab binding.

In forebrain Thy-1 could first be detected at embryonic day (Ed) 8 and the largest increase of Thy-1 levels occurred during the last week of embryonic development which coincides with the major period of neuronal development and synapse formation but precedes the major myelination period.

Thy-1 is first found in the cerebellum and retina at Ed 12. By Ed 18 the cerebellum molecular layer is strongly positive. Heavy particulate staining around the Purkinje cell perikaryal membranes present at Ed 18 is lost in the period to 1 day hatch and is due to the specific loss of perisomatic synapses at this time. A decrease of staining seen in the cerebellar white matter post hatch is due to the masking effect of myelination and not from a loss of Thy-1 from neuronal membranes.

The optic fibre layer and the inner most portion of the inner plexiform layer (IPL) of the retina are positive for Thy-1 at Ed 12. By Ed 18 the staining has progressed across the IPL with 4 lines of stratification being apparent. Day 1 post hatch shows similar pattern but by day 8 post hatch the synaptic stratification of the IPL cannot be seen. No staining was found on the outer plexiform layer further strengthening the retinal ganglion cell localisation for Thy-1. Embryonic spinal cord at Ed 12 and sciatic nerve at Ed 15 express Thy-1 and reach maximum values around 8 days post hatch and the staining diminishes with increasing age due to the masking effect of myelination.

Thy-1 is not transiently expressed on neurons except for the examples of Purkinje cells, spinal cord and sciatic nerve noted here and its role appears to be involved in the processes of the mature, functioning neuron and not in the formation of synaptic connections.

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319.4 BINDING AND INTERNALIZATION OF TETANUS TOXIN TO PC12 CELLS. K. Sandberg and T.B. Rogers. Department of Biological Chemistry, University of Maryland School of Medicine, Baltimore, MD 21201

Tetanus Toxin (T.T.) is a potent neurotoxin which binds selectively to neural tissue. In the present study, the binding of $^{125}$I-T.T. (1-4 x $10^6$ cpm/pmol) to the pheochromocytoma cell line PC12 was examined in order to investigate T.T.'s mechanism of action. The PC12 cells contain complex gangliosides in higher concentrations and bind more $^{125}$I-T.T. than most neuronal cell lines. This is consistent with the evidence that the T.T. receptor is a ganglioside. In this study, the specific binding of $^{125}$I-T.T. was measured on PC12 cells grown in 12 well trays (3.8 $cm^2$/well) and was defined as the total binding minus that occuring in the presence of T.T. antibody (3 units/well). Optimal binding occured in 0.25 M sucrose buffer at pH 7 containing 20 mM Tris, 30 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$ and 0.25% BSA. The cells were incubated with 0.1-0.4 nM $^{125}$I-T.T. (100,000 $^{125}$I-T.T. cpm/well).

The amount of $^{125}$I-T.T. bound to PC12 cells was analyzed as a function of differentiation since various differentiation conditions are known to alter ganglioside composition (Walton, K.M. and Schnaar, R.L., Soc. Neurosci. Abst. 10:760, 1984). PC12 cells were grown under the following conditions: $10^{-5}$M dexamethasone (DEX); 100 ng/ml nerve growth factor (NGF); or, at high density (8 x $10^5$ cells/$cm^2$). Both NGF treatment (582 ± 64 fmol/mg) and high density plating (397 ± 40 fmol/mg) increased the amount of $^{125}$I-T.T. bound per mg protein compared to control cells (263 ± 21 fmol/mg) grown under nondifferentiating conditions ($10^4$ cells/$cm^2$). Dex (283 ± 15 fmol/mg) had an insignificant effect on T.T. binding. These results are interesting in light of the findings that NGF and high density plating have been found to elevate ganglioside levels in these cells compared to control and Dex treated cells (Ibid).

Internalization of $^{125}$I-T.T. (defined as the remaining amount of $^{125}$I-T.T. bound after a 5 min incubation in the presence of 0.4 µg/ml pronase at 37°C) has also been examined. The majority of $^{125}$I-T.T. bound (>60%) to PC12 cells at 0°C is not internalized in contrast to the amount bound (>95%) at 37°C. Most of the bound $^{125}$I-T.T. (>85%) is internalized within 5 min, after the cells incubated at 0°C are transferred to 37°C. While metabolic inhibitors (0.2 µg/ml oligomycin + 0.2 µM rotenone) do not inhibit binding, they do inhibit internalization of $^{125}$I-T.T. (>40%). These experiments demonstrate that $^{125}$I-T.T. binding to PC12 cells is regulated by differentiation conditions and that internalization is dependent upon metabolic energy.

Support by USARMDC contract DAMD17-83-C-3114.

319.5 MONOCLONAL ANTIBODY CAT-301: RECOGNITION OF A SURFACE ANTIGEN ON SUBSETS OF CELLS IN HUMAN CENTRAL VISUAL AREAS AND BIOCHEMICAL PURIFICATION OF THE ANTIGEN FROM GUINEA PIG BRAIN. S. Zaremba*, E. Waldvogel* and S. Hockfield, Cold Spring Harbor Laboratory, P.O. Box 100, Cold Spring Harbor, NY 11724

Monoclonal antibody, Cat-301, recognizes subsets of neurons in central visual areas of cats and monkeys (Hockfield et al., Cold Spring Harbor Symp. 48:877, 1983) recognizing an antigen on surfaces of neuronal cell bodies (Hockfield and McKay, PNAS 80:5758, 1983). We now report on studies to determine whether Cat-301 recognizes cells in human visual areas that may be anatomically and functionally homologous to cells previously identified in cats and monkeys. Such cross-reactivity might permit immunohistochemical analysis of neuronal organization in post-mortem tissue from normal individuals, providing correlation with studies on neuronal organization in experimental animals.

Monoclonal antibody Cat-301 recognizes discrete cell types in human central visual areas and recognizes an antigen on neuronal cell surfaces, a subcellular localization identical to that found in other species. As in the Macaque monkey, antibody positive cells are found in substantial numbers in human primary visual cortex and in fewer numbers in secondary visual cortex. Ongoing experiments are designed to determine whether antibody positive cells are arranged in regular patches when viewed in tangential sections, an arrangement previously shown to reflect the ocular dominance organization in the Macaque. In human lateral geniculate nucleus (LGN), Cat-301 positive cells are found in the magnocellular layers, similar to the distribution previously observed in primates. It is hypothesized that these cells are homologous to the Cat-301 positive cells in cat LGN which previously have been shown to possess anatomical and developmental characteristics of Y-cells.

Biochemical purification of the antigen(s) recognized by Cat-301 is essential to determining whether identical molecules are recognized by the antibody in different brain areas and in different species. Purified antigens are also necessary in order to ask whether the Cat-301 antigen is a member of a multigene family whose members might be expressed specifically in different neuronal subsets. A nitrocellulose dot blot assay has now been set up to screen various tissue extracts for immunoreactivity. Deoxycholate extracts of guinea pig brain stem and spinal cord, concentrated by ultrafiltration, have been shown to possess specific immunoreactivity in amounts amenable to purification. We have been unable to identify Cat-301 antigens on Western blots of deoxycholate extracts known to possess immunoreactivity. Instead, the antigen(s) are being purified from deoxycholate extracts by immunoaffinity chromatography and other biochemical techniques.

This work was supported by NIH Grant RO1 NS18040.

319.6 A MONOCLONAL ANTIBODY AGAINST A NEURONAL CELL SURFACE ANTIGEN. T. Crossfield-Kunze* and S. David. (SPON: A.C.Peterson). Neurosciences Unit, The Montreal General Hospital Research Institute and McGill University, 1650 Cedar Ave., Montreal, Canada, H3G 1A4.

We have generated several monoclonal antibodies using dissociated cells of embryonic day 19 rat corpus callosum as the immunogen. Spleen cells of Balb/c mice immunized with these cells were fused with a Sp2/0 myeloma cell line. Primary wells were screened by an enzyme linked immunosorbent assay (ELISA) and positive wells were cloned by limiting dilutions.

One of these antibodies (2.4A5) binds to a cell surface antigen on neurofilament positive ($NF^+$) cells, as shown by a double indirect immunofluorescence assay on dissociated cell cultures of the neonatal rat cerebral cortex. No other cell types were labelled in these cultures. The labelling of $NF^+$ cells occurs in patches and is more prominent on the processes than over the cell bodies. Indirect immunofluorescence labelling by this antibody of frozen sections of embryonic and early postnatal rat brains fixed in 4% paraformaldehyde, results in faint labelling of neurons in the cerebral cortex and hippocampus.

In preliminary studies in which the developmental changes in this antigen were quantified by an ELISA, a decrease in this antigen was observed after postnatal day 25.

Experiments in which dissociated brain cultures were treated with trypsin or neuraminidase demonstrate that this antigen is sensitive to trypsin but not to neuraminidase.

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319.7 ALTERATIONS IN NEURAL CREST MIGRATION BY A MONOCLONAL ANTIBODY THAT AFFECTS CELL ADHÉSION. M. Bronner-Fraser. Dept. of Physiology and Biophysics, University of California, Irvine, Ca., 92717

Neural crest cells migrate extensively during development along pathways that are lined with extracellular matrix molecules, including fibronectin, hyaluronic acid, and type I collagen. It has been proposed that adhesive interactions between the extracellular matrix and the neural crest cell surface may play a role in neural crest cell migration.

Here, the possible role of a 140kD cell surface complex in neural crest adhesion and migration was examined using a monoclonal antibody JG22, first described by Greve and Gottlieb (1982, J. Cell. Biochem. 18: 221-229). Addition of JG22 to neural crest cells in vitro caused a rapid change in morphology of cells plated on either fibronectin or laminin substrates. The cells became round and phase-bright, often detaching from the dish or forming aggregates of rounded cells. Other tissues such as somites, notochords, and neural tubes were unaffected by the antibody in vitro even though the JG22 antigen is detectable in embryonic tissue sections on the surface of the myotome, neural tube, and notochord.

The effects of the JG22 on neural crest migration in vivo were examined by a new perturbation approach in which both the antibody and the hybridoma cells were microinjected onto neural crest pathways. Hybridoma cells were labelled with a fluorescent cell marker which is non-deleterious and which is preserved after fixation and tissue sectioning. In the mesencephalic region, the JG22 antibody and hybridoma cells caused a marked reduction in cranial neural crest migration in 83% of the injected embryos. The volume occupied by neural crest cells was reduced by about 50% on the injected side relative to the control side. In 26% of the affected embryos, there was a build up of neural crest cells within the lumen of the neural tube. In addition, there was some migration along aberrant pathways. Neural crest migration in the trunk was affected to a much lesser extent. In both cranial and trunk regions, a cell free zone of one or more cell diameters was generally observed between neural crest cells and the JG22 hybridoma cells. Two other monoclonal antibodies, 1-B and 1-N (kindly provided by Dr. C. Buck), were used as controls. Both 1-B and 1-N bind to bands of the 140kD complex recognized by JG22. Neither affects neural crest adhesion in vitro. When these control hybridoma cells and antibodies were microinjected into the mesencephalon, no effects on neural crest migration were observed. This suggests that the observed alterations in neural crest migration are due to functional block of the 140kD complex by JG22. (supported by NIH HD15527-04 and Basic Research Grant 1-896 from the March of Dimes)

319.8 CELL SURFACE LOCALIZATION OF THE LIMBIC ANTIGEN: AN ULTRASTRUCTURAL IMMUNOCYTOCHEMICAL ANALYSIS. P. Levitt and V. Cooper. Dept. of Anatomy, Medical College of Pennsylvania, Philadelphia, PA 19129.

A new type of molecular specificity, expressed among functionally related neurons, was recently discovered with a monoclonal antibody that localized an antigenic determinant within cortical and subcortical regions comprising the limbic system (Levitt, Science 223:299-301, 1984). Immunoperoxidase staining of the antigen at the light microscopic level appeared to be distributed at the cell surface, principally around neuronal somata and within the neuropil. An electron microscopic immunocytochemical study was undertaken to characterize the specific subcellular distribution of the limbic antigen.

Adult Sprague-Dawley rats were perfused transcardially with 4% paraformaldehyde, 0.1% glutaraldehyde and processed for pre-embedding immunoperoxidase staining, applying the limbic system monoclonal antibody to vibratome sections. Semithin and thin sections were collected from medial prefrontal cortex, septum, amydala, hippocampus, nucleus tractus solitarius and lamina II of the spinal cord and the distribution of the antigen on specific cellular elements in each region was examined. Horseradish peroxidase reaction product was found consistently along the cell surface of neurons and their dendritic processes. In all areas examined, neither myelinated nor unmyelinated axons were stained. Thus, the afferent fibers arising from neurons of one limbic region which project to another do not contain immunodetectable antigen. Astrocytes and oligodendrocytes also were unreactive in all regions examined. Within any area, however, all neuronal cell types were stained. For example, hoppocampal pyramidal, granule and polymorphic neurons were immunoreactive as were cells in all layers of prefrontal cortex. Reaction product was distributed in dense clusters that were spaced unevenly along the surface of the somata and large dendritic processes. Only the smallest of dendritic profiles were completely ringed with reaction product. The postsynaptic specializations of some terminal complexes were stained, but most were not immunoreactive. The reaction product that was present within the cytoplasm of stained neurons was usually associated with membrane cisternae, consistent with the transport of the antigen for insertion in the plasma membrane. The presence of the limbic antigen only on the somatic and dendritic surfaces and its absence on axons and axon terminals indicates that the molecule is inserted into the membranes of limbic targets, which is consistent with the antigen's suggested role as a recognition marker among functionally related neurons.

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319.9 COINCIDENT EXPRESSION OF LACTOSERIES CARBOHYDRATES AND β-GALACTOSIDE-SPECIFIC LECTINS BY SUBSETS OF DRG NEURONS. L. Regan*, J. Dodd, S. Barondes and T. Jessell. Department of Neurobiology, Harvard Medical School, Boston, MA. and Department of Psychiatry, University of California, San Diego, CA.

Functionally distinct classes of dorsal root ganglion (DRG) neurons express unique lactoseries and globoseries carbohydrate phenotypes (Jessell & Dodd, Phil.Trans.R.Soc.Lond.B., 308,271,1985). Similar or identical carbohydrate structures have been implicated in cell-cell recognition and adhesion during embryonic development. These observations raise the possibility that cell-surface carbohydrates represent sensory neuron differentiation antigens that contribute to the laminar segregation of sensory terminals in the dorsal horn (DH) of the spinal cord (SC).

The small-diameter DRG neurons that project to the superficial laminae (I,II) of the DH express the N-acetyllactosamine lactoseries backbone structure that is a potential ligand for β-galactoside-specific lectins found in many vertebrate tissues, including the nervous system (Barondes, Science 223,1259,1984). We have examined the possibility that carbohydrate-binding proteins with specificity for N-acetyllactosamine structures are selectively localized and developmentally regulated in DRG and in the DH of the SC, using antibodies raised against two soluble β-galactoside-binding lectins isolated from rat lung. These proteins are biochemically and immunologically distinct, with apparent molecular weights of 14.5Kd and 29Kd (Cerra, Gitt and Barondes, J.Biol.Chem., in press,1985).

Proteins separated by polyacrylamide gel electrophoresis from extracts of DRG and SC were transferred to nitrocellulose paper and probed with the anti-14.5Kd and anti-29Kd antisera. Both DRG and SC extracts contained 14.5Kd and 29Kd immunoreactive proteins. These proteins were the only detectable immunoreactive species in DRG and SC extracts. Sections of adult rat DRG and SC were incubated with anti-29Kd or anti-14.5Kd antisera and processed for immunocytochemistry. The majority of the small-diameter DRG neurons expressed the 29Kd lectin, whereas the large-diameter DRG neurons were not labelled. Most small- and a few large-diameter DRG neurons were labelled by anti-14.5Kd. The central terminals of small DRG neurons in the superficial DH of the SC also expressed the 14.5Kd and 29Kd lectins. Double immunofluorescent staining revealed that all DRG neurons that express the N-acetyllactosamine carbohydrate structure also express the 14.5Kd and 29Kd lectins. The 14.5Kd lectin was detectable in DRG neurons and sensory fibres entering the SC as early as embryonic day (E) 14. By E 18 both the 14.5Kd and the 29Kd lectins were detectable in sensory fibres terminating in the DH. These lectins do not appear to be expressed by DH neurons.

The early and selective expression of β-galactoside-specific lectins by N-acetyllactosamine-bearing sensory afferent terminals suggests a number of strategies for examining the role of both classes of molecules in the development of afferent input to the dorsal horn of the spinal cord.

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## LEARNING AND MEMORY: PHYSIOLOGY IV

320.1 HIPPOCAMPAL STIMULATION DOES NOT IMPAIR SPATIAL REFERENCE MEMORY ACQUISITION. M.L. Shapiro, B.K. Knowlton+, D. Hughey, and D.S. Olton. Department of Psychology, Johns Hopkins University, Baltimore, MD, 21218. +Department of Neuroscience, Stanford University, Stanford, CA, 94305.

The hippocampal system is required for normal memory in humans and non-human animals. However, not all memory processes require the system. This experiment shows that electrical stimulation of the hippocampus that produces hippocampal seizures impairs performance of a working memory task but not the acquisition of a reference memory task.

20 male, albino rats were implanted with bipolar, bilateral stainless steel electrodes in the CA1 layer of the hippocampus. Each rat was placed on the stem of a T-maze and allowed to enter either arm of the maze; only one of the arms contained a food reward. After entering an arm, a rat was treated in one of three ways. The hippocampus was stimulated to produce an electrophysiological seizure (1) immediately after the rat entered the arm (group 1; n=7) or (2) four hours after entering the arm (group 2; n=6), or (3) sham stimulation was given (group 3; n=7). Both the arm containing reward and the type of treatment given to each rat were consistent across trials. Each rat was given one trial per day, and the number of days required to enter the arm containing food reward for five consecutive trials was the acquisition measure of reference memory.

Hippocampal stimulation had no effect on acquiring the reference memory task (mean = 16 days; 1 way ANOVA by groups, P>.90).

The same rats were given hippocampal stimulation while being tested on a working memory task on a 12 arm radial maze. Each arm contained a food reward. Entering each arm without returning to a previously visited arm defined optimal performance. Each rat was forced to 6 arms, given hippocampal stimulation resulting in electrophysiological seizures or sham stimulation, and permitted to choose freely among the 12 arms. Retroactive errors (returning to arms visited prior to seizure) increased from 3% after sham stimulation to 50% after seizure stimulation.

In summary, hippocampal stimulation resulting in seizure activity selectively disrupts performance of working memory tasks but not acquisition of reference memory tasks. These results converge with those from lesion and pharmacological studies to suggest that the hippocampal system is not required for acquiring reference memory information.

320.2 PEAK DISCHARGE RATES OF DENTATE GYRUS NEURONS CORRESPOND TO CHOICE POINTS ON A SPATIAL WORKING MEMORY TASK. B.L. McNaughton, Dept. Psych., University of Colorado, Boulder, CO 80309.

Single and multiple unit activity was recorded in the granule cell layer of the fascia dentata of a rat performing a working-memory task on a radial 8-arm maze. The performance of this task is disrupted by hippocampal damage (Olton et al., Brain Res. 139, 295, 1978). On each trial, the animal was first presented with a random set of four arms. After the food was recovered from the ends of the arms, a one minute delay was interposed, which was followed by presentation of the complete set of eight arms, four of which still contained food reward. The animal performed eight complete trials per recording session, with an intertrial interval of 2 min, and made an average of 1.2 errors per trial. Unit activity was recorded throughout sessions by computer, as was the animal's position at 10 points per second. During the intertrial and intratrial intervals, the most obvious correlate of unit discharge was the animal's movement. This is in agreement with an earlier report by Rose (Neurobiology of the Hippocampus, 449, 1983) which showed that granule cell discharge had a strong movement correlate within a small recording chamber. In contrast, while the animal was actually running the task in the present studies, the peak firing corresponded neither to the animal's peak velocity nor to peak acceleration. Rather, the peak rates were most often associated with the point immediately preceding arm entry. Not infrequently, this peak rate corresponded to a period of reduced velocity or immobility. Occasionally, the animal made one or more partial entries into incorrect arms before making a correct choice. These periods were accompanied by sustained high discharge rates. A similar, but less striking, pattern was also observed in multiple unit records from CA1. While these observations are still open to several interpretations, they do raise the interesting possibility that hippocampal neurons are engaged in particularly intense information processing immediately preceding arm entry.

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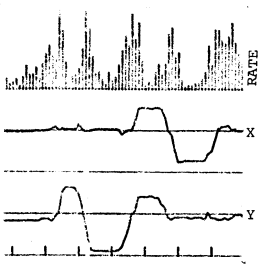


Dentate unit firing rate and corresponding X and Y coordinates while animal was engaged in working memory trial on 8-arm maze. Peak rates correspond to choice points rather than velocity or acceleration maxima. (Time scale 10 sec/div.)

320.3 LONG-TERM STUDIES OF PLACE FIELD INTERRELATIONSHIPS IN DENTATE GYRUS NEURONS. B. Jones Leonard, B.L. McNaughton, C.A. Barnes, Dept. Psych., Univ. Colorado, Boulder, CO 80309.

Several techniques have been reported which permit the discrimination of several spike trains simultaneously from a single probe. The method illustrated here, called the "stereotrode" (McNaughton et al., J. Neurosci. Meth. 8: 391, 1983), is based on the principle that cells with different ratios of distances from two adjacent electrode tips will have different spike-amplitude ratios when recorded on two channels. We report here data from an ensemble of 3 neurons in the fascia dentata (FD) which was monitored over a 17 day period. The study was designed to assess FD place field interrelationships resulting from the removal of distal visual cues while the rat performed forced-choice trials on a radial 8-arm maze.

A male Fischer-344 rat was implanted with a pair of movable stereotrodes which were subsequently positioned in FD. A spike preprocessor calculated 4 parameters for each action potential on each of the X and Y channels. The animal's position on the maze was recorded at 20 Hz by means of a tracking system. Spike parameters, spike event times, and spatial coordinates were stored and later analyzed by means of a cluster analysis program. Three clearly discriminable FD units displayed consistent clusters and reliable place fields on the maze and in the rat's home cage over the 17 days of observation. The experiment reported here consisted of 3 phases during a single recording session. In phase one, the animal was introduced into a darkened room, and 20 trials were collected. Eighteen more trials were run in phase two with the room lights on. During phase three, the room was again darkened, this time in the presence of the rat.

During phase 1, two units exhibited an apparent breakdown of reliable place specificity while a third cell maintained its specificity with respect to maze cues, but shifted this firing to a different arm. In contrast, during phases 2 and 3 all units reverted to the same place fields observed on previous days. These results confirm previous reports by O'Keefe and by Kubie and Muller, showing persistence of place specificity following removal of the determining cues, provided the animal is given an initial spatial reference. They also suggest that individual cells may rearrange their place specificities independently of one another.

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320.4 ACQUISITION OF A CONDITIONED-AVOIDANCE RESPONSE (CAR) IN HYPOPHYSECTOMIZED (HYPOX) RATS AFTER DIFFERENT PERIODS OF RECOVERY FROM THE SURGICAL STRESS. A. Ratner, D.B. Yelvington* and G.K. Weiss.* Department of Physiology, University of New Mexico School of Medicine, Albuquerque, New Mexico 87131.

Acquisition of a condition-avoidance response (CAR) was measured in hypophysectomized (HYPOX) male rats two days, seven days, and three weeks after recovery from the surgical stress. Rats were tested daily in a two-way shuttle box. Testing consisted of ten trials per day with a constant intertrial interval of sixty seconds. A CAR was defined as movement of the rat to the opposite side of the shuttle box within five seconds of presentation of a light (conditioning stimulus - CS). If the animal did not move to the safe side of the box he received a 0.3 ma scrambled shock delivered for fifteen seconds or until he escaped to the opposite side of the box. No significant difference was found in the acquisition of a CAR in SHAM-operated and HYPOX rats given one or three weeks recovery after surgery. However, HYPOX rats allowed only a two day recovery period showed a significantly lower level of acquisition performance when compared to the SHAM rats. The HYPOX rats, after two days of recovery also showed a significant decrease in latency between the presentation of the light (CS) and the avoidance response. Open field observations showed that the motor abilities of the HYPOX rats were not decreased. We were also unable to show any differences between SHAM and HYPOX rats in the thresholds for "any movements", paw movement and the jump-flinch response. These findings indicate that post-surgical recovery time can affect acquisition performance and further suggests that acquisition performance in HYPOX rats may be related to the general physical and metabolic state of the animal.

320.5 NUCLEUS BASALIS INVOLVEMENT IN CONDITIONED NEURONAL RESPONSES IN THE RAT FRONTAL CORTEX. G.C. Rigdon*, W.H. Lyness and J.H. Pirch. Dept. of Pharmacology, Texas Tech University Health Sciences Center, Lubbock, TX 79430.

Neurons in the rat frontal cortex demonstrate altered firing rates in response to conditioned stimuli. Microinjection of inhibitory compounds into the nucleus basalis suppresses the generation of conditioned unit responses in the frontal cortex (Rigdon and Pirch, 1985). Three experiments were designed to test the hypothesis that the basal forebrain cholinergic system is involved in the generation of conditioned cortical neuronal responses in the rat. Most of the cholinergic innervation to the cortex is supplied by projections from the nucleus basalis. A two-second tone cue immediately followed by rewarding medial forebrain bundle stimulation was used to elicit the conditioned neuronal responses in the following experiments. Experiment One was designed to investigate the effects of blocking cholinergic receptors on the conditioned responses of the cortical neurons. Microinjection of atropine, a cholinergic muscarinic receptor antagonist, into the frontal cortex suppressed the conditioned responses of 21 of 24 cortical single units. The nucleus basalis region was lesioned with kainic acid, a neurotoxin, in Experiment Two. The lesion resulted in a significant decrease in choline acetyltransferase activity in the frontal cortex ipsilateral to the nBM lesion. Only 25% of the neurons recorded in the cortex on the side of the nBM lesion exhibited conditioned responses. This was significantly lower than the percentage of neurons that exhibited conditioned responses in the cortex of untreated animals (70%). The firing rates of units in the nBM region were monitored during the cue-event paradigm in Experiment Three. Of the 38 unit recordings from the nBM region, 28 (74%) exhibited conditioned responses. The results from the experiments reported here provide evidence for a role of the basal forebrain cholinergic system in the generation of conditioned single unit responses in the frontal cortex. (Supported by the Tarbox Parkinson's Disease Institute and USPHS MH29653).

320.6 SINGLE UNIT CORRELATES OF CONDITIONING-RELATED TONE DISCRIMINATION IN THE FRONTAL CORTEX OF URETHANE ANESTHETIZED RATS. H.K. Rucker*, M.J. Corbus*, and J.H. Pirch (SPON: P. Meyer). Dept. of Pharmacology, Texas Tech University Health Sciences Center, Lubbock, TX 79430.

This study was designed to ascertain whether neurons in the frontal cortex of urethane anesthetized rats show firing rate changes related to discriminative conditioning. Single unit responses to reinforced (CS+) and non-reinforced (CS-) tone cues were analyzed in 50 neurons that demonstrated a significant response to the CS+ and/or the CS-. Rewarding medial forebrain bundle (MFB) stimulation was used as reinforcement. Prior to the recording session, male adult rats were implanted with MFB stimulating electrodes and allowed to recover. Self-stimulation parameters were obtained and the animals were subsequently trained to associate either a 1KHz or 8KHz tone with MFB stimulation. For unit recording, rats were anesthetized with urethane (1.2 - 1.5 g/Kg, i.p.) and subjected to additional training. Cortical slow potential (SP) responses were recorded with Ag-AgCl electrodes as an index of discrimination (Pirch et al. Intern. J. Neurosci. 25: 207-218, 1985). Extracellular single unit action potentials were recorded with glass microelectrodes. Data acquisition trials were composed of a 2 sec. prestimulus period and two 1000ms response periods following the tone onset, with MFB stimulation applied at the termination of all CS+ tone presentations.

The mean SP response (integrated area) for 18 rats following training under urethane was 79 ± 7 (S.E.) μVsec. for the CS+ and 15 ± 3 μVsec. for the CS- ($p < 0.001$ by paired-$t$). Seventy-eight percent (n=39) of the units analyzed demonstrated an excitatory response to the CS+ significantly greater than the response to the CS-. Sixteen percent (n=8) were inhibited by the CS+; only one unit of this group showed a significant response (inhibition) to the CS-. Six percent (n=3) showed a significant response to the CS- only (two units inhibited and one facilitated). All unit analyses were performed on peristimulus histograms of 25 trials each. Units were frequently held for more than 1.5 hours with stable responses. We conclude that this model represents a stable and reliable in vivo system for studying synaptic mechanisms underlying conditioning-related changes in firing rates of neurons in the rat frontal cortex. (Supported by USPHS MH29653 and the Tarbox Parkinson's Disease Institute at Texas Tech University Health Sciences Center)

320.7 TRYPTOPHAN DIFFERENCES IN SELECTIVELY BRED TASTE-AVERSION PRONE AND TASTE-AVERSION RESISTANT RATS. R. L. Elkins and G. F. Carl. The Med. College of Ga. and the VA Medical Ctr. Augusta GA 30910.

Thirteen generations of selective breeding based on efficiency of taste aversion (TA) acquisition has resulted in significant and marked divergence of strains of TA-prone (TAP) and TA-resistant (TAR) Sprague-Dawley derived rats. During TA conditioning fluid deprived rats consume a novel 0.01% saccharin solution prior to an intraperitoneal cyclophosphamide injection. Aversions are assessed with two-bottle preference tests that provide simultaneous ad lib access to the saccharin solution and plain water. A 15-day percentage preference score is computed for each subject to express the degree to which saccharin solution (CS) ingestion contributed to total fluid intake. These preference scores are the basis of breeder selection. TAP non-sibling breeder pairings are based on the lowest TAP preference scores, and TAR pairings are similarly configured from the highest TAR scores. Strain divergence is an associative phenomena as indicated by standard (pseudoconditioning) control procedures. Separation has been maintained with new CS flavors and modes of illness induction, but has not extended to shock-motivated environmental avoidance learning or to food reinforced bar-press responding.

HPLC analyses of tryptophan and other large neutral amino acids (LNAAs) in blood of TAP and of TAR S-13 males having mean CS preference scores of 2.85 and 95.98, respectively, have revealed lower tryptophan levels in TAP than in TAR rats (mean tryptophan levels = 323.85 and 397.71 µmoles/l, respectively, $p < 0.01$). The ratio of tryptophan to the sum of the other LNAAs in blood is thought to control tryptophan uptake into brain and tryptophan brain concentration (Fernstrom, J. D., Physiological Reviews, 63:484, 1983). This ratio analysis supported lower tryptophan uptake in TAP rats ($p < 0.01$). These findings are consistent with a serotonin influence on TA learning. Tryptophan is a serotonin precursor, and central serotonin depletion has been associated with enhanced TA conditionability (Lorden, J. F. & Margules, D. L., Physiological Psychology, 5:273, 1977).

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320.8 INTERSTIMULUS INTERVAL EFFECTS ON SPINAL CONDITIONING USING A LOW INTENSITY CS. R.G. Durkovic and S.M. Onifer*. Department of Physiology, Upstate Medical Center, Syracuse, NY 13210.

As a simplified neural model of mammalian associative learning, Spinal Conditioning has been shown to exhibit major characteristics of intact animal classically conditioned responding (Behav. Neural Biol. 43:12, 1985). In the spinal preparation it is necessary for the conditioned stimulus (CS) to activate both Aα and Aδ cutaneous fibers in order to demonstrate differences between CS-US paired and CS, US unpaired groups, at least for forward conditioning with a 1 sec interstimulus interval (Exp. Neurol. 86:81, 1984). With such a CS intensity, forward and backward conditioning effects have been observed (Soc. Neurosci. Abstr. 6:191, 1980).

The present study was undertaken to examine the effects of interstimulus interval (ISI) on spinal conditioning using a CS that activated only Aα cutaneous fibers. Ten groups of animals were studied, each with a different ISI. CS alone trials were interspersed among thirty conditioning trials to assess response alterations in all groups. The CS was a 10/s shock to the left saphenous nerve for 1.5 sec. The unconditioned stimulus (US) was a 30/s shock to the left superficial peroneal nerve for 0.5 sec which activated Aα and Aδ fibers of that nerve. Flexion reflex responses to CS presentations were obtained from the tibialis anterior muscle of the same hindlimb.

With simultaneous CS-US onset and with CS onset before US onset no significant differences were observed between the 0, +0.25, +0.5, +1.0, +3.0 sec ISI groups and an explicitly unpaired control group (ISI=30 sec). In contrast, with US onset before CS onset (backward conditioning) significant increases in reflex response to the CS were produced with maximum flexion reflex facilitation with a -0.25 sec ISI and a drop off of facilitation with longer backward ISI's (-0.5, -1.0, -3.0 secs).

The results suggest that the backward conditioning results observed in earlier studies employing a more intense CS were brought about almost exclusively by the Aα cutaneous fiber component of the CS. Such a result differs from the Aδ fiber activation requirement observed for forward conditioning and suggests that in this preparation the neural circuitry involved in backward conditioning is different from that involved in forward conditioning.

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320.9 AN INPUT FROM LOCUS COERULEUS IS NECESSARY FOR DISCHARGE MODIFICATION OF AVIAN LATERAL GENICULATE NEURONS DURING VISUAL LEARNING. J.L. Broyles* and D.H. Cohen. Dept. of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, NY 11794.

We previously reported that a large class of neurons in the avian equivalent of the mammalian dorsal lateral geniculate (LGNe) shows enhancement of light (CS)-evoked responses over visual conditioning. This class consists of cells showing decreased discharge in response to the unconditioned stimulus (US), foot-shock. Cells with US-evoked increases in discharge show habituation of their CS-evoked responses. These findings indicated that CS-US convergence is necessary for associative modification of LGNe discharge. However, such convergence is not sufficient, since the US input must have specific properties, in contrast to the CS input. We subsequently reported that a projection from the locus coeruleus (LC) to LGNe relays the US input responsible for decreased discharge of LGNe cells. The present experiments were designed to evaluate whether eliminating this US input by interruption of the coeruleo-LGNe pathway also eliminates the modifiability of LGNe neurons.

The activity of single LGNe cells with US-evoked decreases in discharge was isolated. After 5 unpaired CS and US presentations, an electrolytic lesion was made in the ipsilateral coeruleo-LGNe pathway. Another block of unpaired CS and US presentations was then given, followed by paired CS-US presentations. Consistent with previous findings, the lesion changed US-responsiveness in 81% of the cells. In 27% the US-evoked response transformed to increased discharge; 45% showed less decrease in discharge; and 9% became unresponsive. Of the cells held for at least 15 conditioning trials, only 9% showed any enhancement of their CS-evoked response, and this was of much lower magnitude than in intact animals. The lesion did not affect CS-evoked responses. It appeared to decrease maintained activity, but the effect was not statistically significant.

In very preliminary experiments, a lesion was made in the contralateral coeruleo-LGNe pathway. Although the coeruleo-LGNe projection is bilateral, it is predominantly ipsilateral. Therefore, animals with lesions of the contralateral pathway constitute an interesting control. The few cells studied to date showed response enhancement over conditioning, but of a lower magnitude than usually seen in intact birds.

These experiments show that the coeruleo-LGNe pathway is necessary for associative modification of LGNe neurons. Since another study showed that electrical stimulation of LC is effective as a US, we conclude that the LC input is both necessary and sufficient for transmitting effective US input to LGNe.

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320.10 A NEW HYPOTHESIS FOR THE NEURAL BASIS OF ANESTHESIA. S. A. Raymond. Anesthesia Research Laboratories, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115

Several mechanisms have been proposed for the general anesthesia induced by various pharmacological agents. 1) excitatory synaptic connections may be "blocked". 2) The action of inhibitory synapses for presynaptic inhibition may be enhanced. 3) conduction may fail presynaptically at regions of relatively lower conduction safety along axons or in axonal teledendra. Or as suggested below, 4) activity-dependent processes in neuronal membranes may be disturbed.

The action of local anesthetics (chloroprocaine, lidocaine, bupivacaine), volatile inhalational agents (halothane and nitrous oxide) and general anesthetics administered by injection (ketamine) has been studied on single frog sciatic axons in vitro. The threshold, conduction latency, and waveform of the action potential recorded through suction electrodes were monitored as a function of anesthetic concentration over the range in which anesthesia occurs in amphibians, mice, and humans. For the several agents studied to date, it has been consistently observed that activity-dependent processes are inhibited at concentrations 3 to 5 times lower than those required to elevate resting threshold, to slow conduction or to diminish the action potential by an equivalent percentage. Superexcitability is a lowering of threshold by 5 to 50% that reaches a maximum within 20 msec following impulse discharge and recovers to baseline over about 1 sec. The magnitude and duration of superexcitability following single impulses or trains of 8 pulses was reduced by all agents at anesthetic concentrations. Similarly depression, a phase of raised threshold which builds up as activity rates exceed 1 pulse every 2 sec and recovers over several minutes to an hour or more following a period of activity at 2-4 Hz, became undetectable at concentrations 1/3 to 1/10 those required to block impulse conduction. Individual fibers showed marked variations in susceptibility, but reduced activity-dependence was consistently the first detectable event associated with the presence of the drug.

Even under conditions where anatomical connectivity as tested by reflexes is not altered by the anesthetic, a reduced activity-dependence may disrupt the normal relation between patterns of impulse activity and dynamic connectivity of the nervous system, leading to the incapacity to make sense of environmental stimuli. The hypothesis that disruption of activity-dependent changes in nerve membrane is the basis of general anesthesia makes the testable prediction that an agent that diminishes activity-dependence should produce unconsciousness and prevent short term memory even if it does not suppress or block impulse conduction.

320.11 TEMPORAL ORDER AND BLOCKING IN A NEURAL NETWORK MODEL OF CLASSICAL CONDITIONING. D.S. Levine, Department of Mathematics, University of Texas at Arlington, Arlington, TX 76019.

This numerical work tests a classical conditioning model based on a neural network with associative ("Hebbian") modifiable synapses. It has been claimed (Sutton, R. and Barto, A., Psych.Rev. 88: 135,1981) that such a model could not simultaneously explain two sets of data: that the conditioned response (CR) precedes (anticipates) the unconditioned stimulus (US), and that the optimal time interval between conditioned stimulus (CS) and US is larger than zero. The argument was that the optimal CS-US interval in a network with associative synapses will be zero unless the network includes a certain delay, and this delay will prevent the CR from preceding the US. To overcome this difficulty, these authors proposed a more elaborate synaptic modification rule and two traces for each stimulus.

Computer simulations were done to test an alternative neural explanation for these data, one which uses the "Hebbian" rule and involves no delays. The poor conditioning when CS and US are less than 500 msec apart was seen as an example of blocking, where one stimulus is masked by another, more salient, stimulus. In this case, the US itself blocks the CS. A model neural network with associative synapses (Grossberg, S., Int. Rev. Neurobiol. 18: 263, 1975) was shown capable of reproducing both the anticipatory CR and the non-zero optimal CS-US interval. The same network could also reproduce conventional blocking data: first, $CS_1$ is conditioned to the US; then, $CS_1$ and $CS_2$ are presented together repeatedly, followed each time by the US; finally, $CS_2$ is presented and no CR occurs.

320.12 NEURAL DYNAMICS OF ADAPTIVE PATTERN RECOGNITION: PRIMING, SEARCH, ATTENTION, AND CATEGORY FORMATION. G.A. Carpenter* and S. Grossberg. Center for Adaptive Systems, Boston Univ., Boston, MA 02215.

We show how a neural network can learn recognition categories by building up bottom-up codes and top-down expectancies in response to a temporal stream of input patterns. The internal representations formed in this way stabilize themselves against recoding in response to irrelevant input patterns by using the matching properties of the learned top-down expectancies. This code-stabilizing mechanism also suppresses noise in the input patterns, and can subliminally prime a network to anticipate an input pattern or class of input patterns.

A nonspecific vigilance, or attentional, parameter determines how fine the learned categories will be. If, for example, vigilance increases due to a negative reinforcement, then the system will automatically search for and learn finer recognition categories. Although a novel input pattern may automatically engage the top-down matching and parallel search mechanisms of the network, once a recognition category for the input pattern is established, the input pattern can access the category directly on future recognition trials.

This search mechanism is not a serial mechanism. Rather, it is driven by the engagement of a nonspecific orienting burst that is contingent upon a prior mismatch event. This orienting reaction has been used to explain and predict properties of the mismatch negativity, processing negativity, and P300 evoked potentials and noradrenergic involvement in cortical plasticity.

The model's flexible and dynamic relationship between matching, orienting, attention, and learning proves its worth by very rapidly learning and self-stabilizing perceptual categories of any prescribed refinement. The coarseness of the categories is in no respect prewired, nor is an identity match performed. In fact, the learned top-down expectancies become more abstract as the categories become broader. The learned code is an emergent property of the total network response, given a prescribed level of attentional tuning.

## LEARNING AND MEMORY: ANATOMY IV

321.1 MORPHOLOGICAL BASIS OF SHORT-TERM HABITUATION IN APLYSIA. C.H. Bailey and M. Chen. Ctr. for Neurobiol. & Behav., Depts. Anat., Neurol. and Psychiat., Columbia Univ., P&S, and NYS Psychiat. Instit., N.Y., N.Y. 10032.

To complement our ultrastructural studies of long-term habituation and sensitization in Aplysia (Science, 220:91), we have begun a quantitative analysis of the structural events at identified sensory neuron synapses that may accompany a short-term memory trace.

Utilizing an in vitro preparation, we have labeled identified synapses by intrasomatic injections of small amounts of HRP while simultaneously producing short-term habituation in individual cells employing single, massed training protocols of either 1-35 (isi=30 secs) or 1-250 (isi= 10 secs) stimuli. The sensory-to-follower cell EPSP was monitored throughout and in all cases demonstrated significant depression (compared to trial 1) at the end of training. Immediately following the last stimulus, the desheathed abdominal ganglion was fixed with 6% glutaraldehyde. Parallel ganglia treated exactly the same but without training served as controls. Following histochemical processing, postfixation and embedding, serial thin sections were taken through each ganglion and labeled sensory neuron varicosities were reconstructed and analyzed through a blind procedure. Eight sensory neurons from eight different animals, 4 untrained cells as controls and 4 cells trained for short-term habituation, were used in this study. To examine in detail the fine structure of synaptic terminals of each cell, we completely reconstructed 422 sensory neuron varicosities.

We focused our analysis, as we had in our long-term studies, on the active zones at sensory neuron varicosities. Both the incidence as well as the total surface area of active zones were unchanged between control and habituated cells, although habituated cells in the 1-250 group did show a trend towards reduced active zone area compared with their controls. To facilitate an analysis of the relationship between the active zone and neighboring synaptic vesicles, we divided the volume of the sensory neuron varicosity immediately above each active zone into different sectors. The total vesicle population was defined as all vesicles within a vertical distance of 240 nm (the approximate height of 3 vesicles) over the entire active zone area. A readily releasable pool was defined as all vesicles that came within 30 nm of the presynaptic active zone membrane. At habituated terminals the releasable pool was half that found at control terminals (7.6 $\pm$ 0.6 S.E.M. vs. 14 $\pm$ 0.8, 1-35 and 6.7 $\pm$ 1.4 vs. 15.8 $\pm$ 1.6, 1-250). The ratio of releasable to total vesicles was .26 and .30 for control cells but only .11 at the terminals of habituated cells, suggesting that an impairment in vesicle mobilization at sensory neuron active zones may contribute, in part, to the synaptic decrement that underlies homosynaptic depression.

These data indicate that a short-term memory trace can be specified in morphological terms and provide additional insights into the family of structural changes at sensory neuron synapses that may contribute to the progressive development of memory storage.

321.2 LONG-TERM HABITUATION OF ACOUSTIC STARTLE AND LICK SUPPRESSION FOLLOWING LESIONS OF THE CEREBELLAR VERMIS. R. N. Leaton and W. F. Supple, Jr. Department of Psychology, Dartmouth College, Hanover, N.H. 03755.

Mechanisms for short-term habituation of the acoustic startle response are apparently intrinsic to the S-R pathway in the lower brainstem and involve some form of synaptic depression within that pathway (Davis et al., 1982; Thompson & Spencer, 1966). Long-term habituation in this response system seems to involve an extrinsic inhibitory mechanism (Jordan & Leaton, 1983), but little is known of its anatomical locus. We have recently shown (Supple & Leaton, 1984) that the vermis of the cerebellum is essential for long-term habituation of the acoustic startle response. The present experiments extend these earlier findings (1) by examining the response specificity of the effects and (2) by testing at two stimulus intensities to avoid confounding floor and ceiling effects.

In Experiment 1 the acoustic startle response and lick suppression were measured simultaneously to 1-sec, 10-kHz pure tone stimuli in rats with partial aspirations of the cerebellar vermis (n=10) and sham operated rats (n=10). Six stimuli were presented on an approximately 10-sec ISI in each of 5 daily sessions. Long-term habituation was assessed by the response decrements across days. The vermis lesioned rats failed to show long-term habituation of the acoustic startle response, replicating our previous findings. Both groups showed significant long-term habituation of lick suppression. Thus the effects of vermal lesions on long-term habituation are at least partially response specific.

In Experiment 2 independent groups of vermal lesioned and sham operated rats were tested at two intensities of a 1-sec white-noise startle stimulus (97 or 107 dB). Testing was given in two one-trial sessions on each of 8 days and retests were given following tests for short-term habituation. Vermal lesioned animals failed to show significant long-term habituation at either test intensity. Initial response levels and short-term habituation were not affected by the vermal lesions. Activity samples taken during the startle sessions revealed no differences between vermal lesioned and control groups. The conclusion that vermal lesions block long-term habituation of the acoustic startle response is not confounded by floor or ceiling effects or by differences in general activity during startle sessions.

321.3 **INTRINSIC NEURONS IN THE AMYGDALOID FIELD INNERVATED BY THE MEDIAL GENICULATE NUCLEUS MEDIATE CLASSICAL FEAR CONDITIONING IN THE RAT.** J. Iwata, J.E. LeDoux, D.J Reis. Lab of Neurobiology, Cornell Univ. Med. Coll., NY, NY 10021

Although the auditory cortex is generally believed to be the principal efferent target of the medial geniculate nucleus (MG), we have found that neurons in the MG also project to a striatal field (STR) involving the caudal neostriatum (caudate-putamen; CPU) and archistriatum (dorsal amygdala; AMY) (LeDoux et al, J. Neurosci. 4: 683-698, 1984; LeDoux et al, this vol.). Interruption of these connections by electrolytic lesion of one MG and the contralateral STR disrupts the conditioning of emotional responses to acoustic stimuli (LeDoux et al, Ann. N.Y. Acad. Sci., in press). Destruction of intrinsic neurons in STR by injection of ibotenic acid (IBO) also disrupts conditioning when the contralateral MG is lesioned (Iwata et al, Neurosci. Abs. 1984). In the present study we have sought to determine whether conditioning is mediated by intrinsic neurons in the AMY or CPU portion of STR.

Rats were prepared with unilateral electrolytic lesions of MG. Contralaterally, IBO (0.15 ul; 15 ug/ul) was injected into the posterior CPU (n=11) or dorsal AMY (n=23). Controls were unoperated (n=16) or received unilateral MG lesions with vehicle injections (0.3ul of 0.2M phosphate buffer; ph, 7.4) in the center of the contralateral STR (n=13). After 14 d the animals were instrumented for computer assisted recording of arterial pressure (AP) and subjected to classical conditioning procedures involving the pairing of a pure tone (800 Hz, 82 db, 10 sec) with footshock (1.7 mA; 0.5 s). The next day CS elicited changes in AP and the duration of activity suppression ("freezing") were measured during extinction trials presented in the animal's home cage. The animals were sacrificed and the brains removed and processed using standard histological procedures. The lesions were reconstructed and localized using a computer based image analysis system.

In unoperated controls the CS elicited increases in AP (20 ± 3 mmHg) and induced freezing (106 ± 3 sec). These responses were unaffected by MG lesions combined with injection of vehicle in the STR or combined with injection of IBO in the CPU. Lesion of the MG combined with injection of IBO in the contralateral amygdala had no effect in 12 rats but in 11 additional rats reduced both the AP (6 ± 1 mmHg) and freezing (30 ± 8 sec) responses by more than 2 standard deviations relative to the vehicle control group.

Computer analysis of lesion location revealed that the effective AMY lesions consistently damaged dorsal portions of the central nucleus and the overlying fundus striati. Ineffective lesions overlapped the location of effective lesions but occupied far less volume (effective: 2.14 ± 0.64 $mm^3$; ineffective: 0.64 ± 0.15 $mm^3$; p .01). CPU lesions (2.39 ± 0.37 $mm^3$) were similar in size to effective AMY lesions.

Thus, selective destruction of intrinsic neurons in the amygdaloid field innervated by the MG disrupts fear conditioning when the contralateral MG is destroyed. Fear conditioning is therefore medidated by projections from MG to the amygdala. (Support: Am. Heart Assoc. and NIH-NHLBI)

321.4 PARASAGGITAL KNIFE CUTS LATERAL TO THE BED NUCLEUS OF THE STRIA TERMINALIS AND MEDIAL TO THE AMYGDALA SEVERELY ATTENUATE THE CARDIAC ORIENTING REFLEX AND CLASSICALLY CONDITIONED BRADYCARDIA IN THE RABBIT. Paula Skedsvold* and D. A. Powell (SPON: J. Freeman). Neuroscience Lab., WJB Dorn VA Hospital and University of S. C., Columbia, SC

Using a Kopf Scouten knife assembly one group of New Zealand albino rabbits received bilateral knife cuts which were 2 mm from the midline and extended from 1-2 mm in front of the anterior commissure to a posterior location just in front of the anterior preoptic area. A second group received sham cuts in which the knife assemby was lowered into the brain but no cut was made. After a 1-2 wk recovery period all animals were subjected to a 30 min session in which the cardiac orienting reflex (OR) was assessed and 2 subsequent sessions of classical conditioning in which heart rate (HR) was assessed on a beat x beat basis. Orienting was assessed in response to successive presentations of twenty 75 dB, 1216 Hz, 4-sec duration tones separated by a 90-sec intertrial interval. HR was assessed for 10 beats prior to tone onset, for the entire duration of the 4-sec tone, and for 10 beats following tone offset. The first day of classical conditioning began immediately followed the assessment of the cardiac component of the OR. During conditioning 25 presentations of the 1216 Hz tone and 25 presentations of a 304 Hz tone were presented in a random order. One of the tones was consistantly followed by a 3 mA paraorbital electric shock as the unconditioned stimulus. HR was assessed as during orienting. A third group of sham animals received a random sequence of the tones and shocks, viz., the tones and shocks were not paired as during classical conditioning. This group served as a pseudoconditioning control group.

The results revealed that animals which received parasaggital knife cuts, as described above, showed very little HR change in response to either CS+ or CS- compared to sham animals which revealed HR slowing as the conditioned response. Animals with knife cuts also revealed an attenuated bradycardiac OR compared to sham animals. These cuts severed fibers comprising the sublenticular and posterior internal capsule carrying information from prefrontal cortex, insular cortex, and amygdaloid structures which have been implicated in autonomic control. Together with other lesion and stimulation studies from our laboratory, the present findings suggest that these forebrain structures are involved in a sensory processing mechanism which elicits primary bradycardia in response to either novel stimuli or nonaversive stimuli that have been consistently reinforced with an aversive stimulus and thus come to serve as signals for noxious stimulation.

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321.5 AMYGDALA CENTRAL NUCLEUS AS MEDIATOR OF DIFFERENTIAL PAVLOVIAN CONDITIONING OF BRADYCARDIA IN RABBITS. C.G. Gentile*, T.W. Jarrell*, A.H. Teich*, P.M. McCabe and N. Schneiderman. Dept. of Psych., Univ. of Miami, Coral Gables, FL 33124.

The present study examined the role of amygdala central nucleus (ACE) in the retention of differential Pavlovian conditioning of bradycardia in rabbits. It has been demonstrated that ACE lesions produce a marked impairment in the acquisition of the bradycardia conditioned response (Kapp et al., Physiol. Behav., 23:1109, 1979). Furthermore, auditory stimuli paired with eye shock elicit different patterns of ACE single unit firing than unpaired stimuli (Pascoe and Kapp, Neurosci. Abs., 10:602, 1984). These data suggest that ACE may mediate differential bradycardia conditioned responses.

Electrodes were implanted bilaterally in ACE or in control sites just dorsal and rostral to ACE. Two days following surgery, animals were subjected to differential Pavlovian conditioning in which one tone (CS+) was paired with periorbital shock and a second tone (CS-) was presented alone. Each animal received one conditioning session per day until evidence of differential heart rate (HR) responding was obtained. Bilateral electrolytic lesions were then made. Thirty minutes after lesioning, animals received an additional conditioning session.

Both control and ACE groups demonstrated differential HR responses prior to lesioning. In the control group, lesions had no effect on differential HR responses or bradycardia response magnitude. However, the ACE lesion group failed to demonstrate differential HR responses after lesioning. Furthermore, bradycardia conditioned response magnitude was greatly attenuated. In both groups, lesions had no effect on the HR orienting response, unconditioned response, or baseline.

These findings suggest that ACE plays a role in the retention of differential Pavlovian conditioning of bradycardia in rabbits. Bilateral ACE lesions abolished differential HR responses and profoundly attenuated bradycardia response magnitude.

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321.6 THE MEDIAL GENICULATE NUCLEUS AS MEDIATOR OF DIFFERENTIAL PAVLOVIAN CONDITIONING OF BRADYCARDIA IN RABBITS. T.W. Jarrell*, C.G. Gentile*, P.M. McCabe, and N. Schneiderman. Dept. of Psych., Univ. of Miami, Coral Gables, FL 33124.

The present study examined the role of the medial geniculate nucleus (MGN) in the mediation of differentially conditioned bradycardia responses to auditory stimuli in rabbits. Kapp et al. (Physiol. Behav., 23:1109, 1979) provide evidence that the central nucleus of the amygdala (ACE) plays an important role in the acquisition of classically conditioned bradycardia in rabbits. In addition, there is evidence that an auditory stimulus that has previously been paired with eyelid shock elicits different patterns of ACE unit firing than one that has not been paired with shock (Pascoe and Kapp, Neurosci. Abs., 10:602, 1984). These data suggest that ACE may receive information about auditory conditioned stimuli that is important for differential responding.

Intial injections of horseradish peroxidase into the ACE produced cell body and fiber labeling at the ventral and medial borders of MGN. The role of this region in the mediation of differential Pavlovian conditioning of heart rate (HR) decelerations and corneo-retinal potential (CRP) responses was then examined. Bilateral electrolytic lesions were made in the medial portion of MGN or in control sites dorsal or rostral to MGN. Ten days following surgery, lesioned animals and unoperated control animals were subjected to 7 days (1 session/day) of differential Pavlovian conditioning consisting of trials in which one tone (CS+) was paired with periorbital shock and a second tone (CS-) was presented alone. The 7 acquisition days were followed by 2 days of extinction in which both tones were presented alone.

Each group demonstrated bradycardia responses to both the CS+ and CS-. In the control-lesion and unoperated groups, the CS+ consistently elicited larger bradycardia responses than the CS-. However, animals with bilateral MGN lesions did not demonstrate differential bradycardia responses. In the MGN group, bradycardia responses to both the CS+ and CS- were similar in magnitude to responses to the CS- in the control-lesion and unoperated groups. Evidence of CRP differential conditioning was present in each group.

The present findings suggest that a region just medial to MGN or fibers passing through this region selectively mediate HR differential conditioning in rabbits. The fact that bradycardia responses are still present after lesions at the medial border of MGN suggests that other auditory regions may also be involved in the mediation of the bradycardia conditioned response.

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321.7 THE SEPTO-HIPPOCAMPAL ROLE IN THE ACQUISITION OF TASTE POTENTIATED ODOR AVERSION LEARNING. F. Bermudez-Rattoni, G. Roldan*, M.A. Sanchez* and M.L. Marquez. Departmento de Fisiologia. Centro de Investigaciones en Fisiologia Celular, Universidad Nacional Autonoma de Mexico.

In flavor-aversion conditioning, odor (O) and taste (T) display different parameters of conditioning. O must be paired with immediate toxic injections but T can be followed by delayed toxin. However, when an OT compound is followed by delayed toxin, the O component becomes aversive indicating it has been potentiated by the T component.

In testing a neural model for the potentiation of O by T, it was shown that "reversible" and irreversible lesions of the amygdaloid complex disrupts potentiated O aversions but not T aversions. Moreover, we have demonstrated that the cholinergic limbic system is apparently involved in the potentiation of O by T in toxic conditioning. Thus agonists and antagonists of cholinergic activity, applied in the dorsal hippocampus or amygdala affect O-toxin associations more than T-toxin associations. In the present experiment, the role of the septal area and the doral hippocampus were tested in the conditioning of potentiated odor by taste aversions. Four groups of rats were given large electrolytical lesions, bilaterally in the dorsal hippocampus (BH), unilaterally in the dorsal hippocampus (UH), bilaterally in the septal area including medial and lateral areas (S) and sham operations (SH). Following postoperative recovery, each group received OT acquisition; almond odor and saccharin were combined into a drinking solution and followed by delayed lithium chloride (190 mg/kg, i.g.).

Extinction tests with almond odor alone and saccharin alone showed that the SH group developed strong O and T aversions, however S and BH lesion groups showed a significant disruption of the O aversion when compared to the SH controls and UH groups. (All groups exhibited strong taste aversions.)

These preliminary results suggested that the septo-hippocampal system are particularly involved in the process by which the odor is indexed into the visceral system.

321.8 DISRUPTION OF NEOPHOBIA, CONDITIONED ODOR AVERSION AND CONDITIONED TASTE AVERSION IN RATS WITH HIPPOCAMPAL LESIONS. J.S. Miller*, A.J. Nonneman, K.S. Kelly*, J.L. Neisewander* and W.L. Isaac*. Psychology Dept., Univ. of Kentucky, Lexington, KY 40506.

Many studies have implicated limbic system structures, including the hippocampus (HPC), in the control of flavor aversion learning. Flavor aversion conditioning consists of pairing a distinctive flavor (CS) with illness (US). Since flavor consists of both odor (O) and taste (T) components, this aversion may develop to either the O or T elements of a flavor. Few studies have examined the role of the hippocampus in odor aversion learning, but physostigmine injections into the dorsal HPC disrupt conditioned odor aversions (Bermudez-Rattoni & Garcia, Soc. Neurosci. Abstr., 10:256, 1984). The effects of HPC lesions on taste aversion conditioning seem to depend on the strength of conditioning (Nonneman & Curtis, Physiol. Psychol., 6:249-256, 1978). Weakly conditioned T-illness associations are disrupted and strongly conditioned associations remain unaffected.

Relative to T, O serves as a weak cue for conditioning aversions. However, when O and T are conditioned in compound, the subsequent aversion to the odor cue alone is often stronger than if O had been conditioned alone. This phenomenon is known as taste potentiated odor aversion. In this study we tested the hypothesis that HPC lesions would disrupt taste potentiated odor aversions less than odor aversions.

Each rat received a bilateral electrolytic HPC or sham lesion. During conditioning, half the rats in each group were exposed to the compound O-T CS (1.5% almond extract + .1% saccharin) for 15 min. followed by an i.p. injection of LiCl (64 mg/kg) or saline. The other half was exposed to the O CS alone followed by LiCl or saline. On test days, all rats were exposed to the O CS alone to assess the strength of learned odor aversions. On the final test day the rats that had been conditioned to the O-T compound were exposed to the T CS (.1% saccharin) alone to determine the strength of any acquired taste aversion. The HPC lesions eliminated the neophobic response to the O CS and significantly reduced neophobia to the O-T CS on the first CS exposure. The lesions also eliminated conditioned odor aversion and significantly attenuated acquired taste aversion.

321.9 THE EFFECT OF MATERNAL BEHAVIOR SENSITIZATION OR EXPOSURE TO PUPS ON PROTEIN PHOSPHORYLATION IN THE FEMALE RAT AMYGDALA. P.J. Brooks*, B. Bank*, H. Steiman* and D.L. Chute. Department of Psychology and the Division of Life Sciences, University of Toronto, Toronto, Ontario, Canada M1C 1A4.

Work in this laboratory has focused on the relationship between behavioral plasticity and protein phosphorylation in the brain. The purpose of the present study was to determine whether changes in protein phosphorylation could be demonstrated as a result of maternal behavior sensitization (MBS). MBS is the phenomenon in which virgin female rats, which initially avoid newborn pups, will come to show maternal behavior following several days of pup exposure. An additional aim of this work was to determine the effect of pup exposure alone on brain protein phosphorylation.

Twelve female rats were cast into three behavioral groups (n= 4 per group). One group received no pup exposure and served as a control (group C). The remaining subjects were tested daily for maternal behavior using three rat pups (2-8 days old). Four animals were allowed pup exposure until they became fully maternal (sensitized-group S), while pups were removed from the other four animals before any maternal behavior was exhibited (pup exposed-group E). Rats were killed by decapitation, the heads were immediately frozen in liquid $N_2$ and stored at -70 until biochemical processing. Based on lesion studies suggesting the importance of the medial preoptic area (MPOA) and corticomedial amygdala (CMA) in MBS[1], these areas were dissected out along with a portion of frontal cortex (FC) as a control brain area. Tissues from all animals in a behavioral group were pooled and homogenized. Samples from the different brain areas were incubated with $\gamma P^{32}$-ATP and analyzed using SDS-PAGE followed by autoradiography and optical density scanning.

No reliable between group differences were seen in the MPOA or FC. In the CMA, $P^{32}$ incorporation into a protein band of Mr 23K was 2-3 fold greater in groups S and E compared to group C. No differences were seen between groups S and E in the CMA.

These results suggest that exposure of female rats to pups can result in phosphorylational changes in the brain similar to those seen in several learning and neuroplastic paradigms. Further work is needed to determine the relationship between the phosphorylation changes seen in the study and the behavioral changes which occur in MBS.

[1]Fleming et al. Phys. & Behav. 31:503.

321.10 CEREBELLAR INTERPOSITUS/DENTATE NUCLEI AFFERENTS SEEN WITH RETROGRADE FLUORESCENT TRACERS IN THE RABBIT. J.K. Thompson*, D.G. Lavond & R.F. Thompson. Dept of Psychology, Stanford University, Stanford, CA 94305.

Recent behavioral experiments have demonstrated an essential role of the deep cerebellar nuclei in learning and memory. Lesions of the nuclei prevent or abolish classical conditioning of the rabbit eyelid response without affecting the unlearned reflexive response caused by corneal airpuff. Evidence suggests that the cerebellum may be the site of neuronal plasticity associated with this learning and memory--the "engram". Lesions of the climbing fiber (inferior olive) input prevent learning or cause extinction of an already learned response. Direct stimulation of the dorsolateral pons or of the lateral reticular nucleus act as effective conditioned stimuli while stimulation of the IO acts as an effective unconditioned stimulus.

The present experiment was designed to survey the possible routes of auditory input to the deep nuclei. The route of a naturally occuring CS (i.e., tone) is uncertain. A total of 26 rabbits were anesthetized and stereotaxically injected with fluorescent tracers into the deep nuclei (N=24) or overlying cerebellar cortex (N=2). The dyes used included bisbenzimide, propidium iodide, Evans blue, True Blue, Nuclear Yellow and Fast Blue. The rabbits were allowed to survive for 18 hours to 3 weeks depending upon the transport rate of the dye. Most rabbits were injected with a different fluorescing dye on each side.

Labelled cells were commonly found within the inferior olive contralaterally, the pontine nuclei bilaterally, the trigeminal sensory and spinal nuclei bilaterally, and the lateral reticular nucleus bilaterally. In addition, cells were labeled at many levels throughout the reticular formation, in the perihypoglossal nuclei, in nucleus ambiguous, in the raphe, in the cuneate nucleus, in vestibular nuclei, in the red nucleus contralaterally, and occassionally in the nuclei surrounding the superior olive.

There was a noticeable absence of direct auditory projections from the cochlear nuclei, the superior olive, the trapezoid nucleus, and the nuclei of the lateral lemniscus. Double labelled cells were found in the lateral reticular, reticular, and trigeminal nuclei. A few ipsilateral cells were found in the IO and in the VIIIth nerve. A number of labelled cells were found in the contralateral cerebellum. More lateral injection of the nuclei resulted in predominantly ipsilateral labelling in trigeminal nuclei, and less labeling of dorsolateral pons and the lateral reticular nucleus.

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321.11 A COMPARISON OF CEREBRAL ACETYLCHOLINE OR SOMATOSTATIN DEPLETION ON AVOIDANCE CONDITIONING, ACTIVITY AND WORKING MEMORY IN RATS. G.R. Sessions, M.D. Matthews*, N. Swerdlow, C. Bakhit* and G.F. Koob. Department of Behavioral Sciences and Leadership, U.S. Air Force Academy, Colorado Springs, CO 80840, and Division of Preclinical Neuroscience and Endocrinology, Scripps Clinic and Research Foundation, La Jolla, CA 92037.

Decreases in cortical achetylcholine (ACh) and degeneration of cholinergic neurons of the nucleus basalis of Meynert have been linked with senile dementia of the Alzheimer's type. Decreases in cortical somatostatin have also been observed in Alzheimer's patients. Lesions of the nucleus basalis magnocellularis (NBM) in the ventromedial region of the globus pallidus of the rat have been shown to reliably deplete markers for cortical ACh. Intracerebroventricular (ICV) injections of cysteamine have similarly been demonstrated to reliably deplete hypothalamus, hippocampus, and cortex of somatostatin. Both of these procedures have been shown to induce deficits in passive avoidance retention in rats. In an effort to further the investigation of the usefulness of animal models in the study of Alzheimer's Disease, we compared the effects of depletion of cerebral ACh or somatostatin on tasks involving learning, working memory, and activity in the rat.

Rats received bilateral lesions of the NBM via intracerebral injections of ibotenic acid or depletion of somatostatin by ICV administration of cysteamine. Activity testing and active avoidance conditioning were conducted in a two-compartment shuttle box. The active avoidance task involved a one-way procedure utilizing a light CS. The test of working or short-term memory involved measuring spontaneous alternations in a Y-maze.

Results revealed that the NBM-lesioned animals were significantly more active in the shuttlebox, exhibited significantly fewer alternations in the Y-maze, but showed no differences in active avoidance acquisition as compared with their controls. The cysteamine-injected animals sustained significant depletions of somatostatin in cortex, hippocampus and hypothalamus (25-59 per cent) but showed no deficits on any of these measures.

These results are consistent with previous work showing increased activity and working memory deficits in rats with lesions of the NBM. Findings suggesting deficits in active avoidance conditioning were not supported. Failure to obtain deficits in somatostatin-depleted animals on measures of working memory and active avoidance acquisition suggests that memory deficits observed in such animals may be limited to consolidation processes.

321.12 EFFECTS OF TRAINING ON A REACHING TASK ON DENDRITIC BRANCHING IN THE NEOCORTICAL REGION OF HIGHEST METABOLIC ACTIVITY DURING REACHING PERFORMANCE. G. S. Withers, J. R. Larson, and W. T. Greenough. (Spon: S. Balagura) Depts. Psychol. & Anat. Sci. and Neur. & Behav. Biol. Prog., Univ. Illinos, Urbana-Champaign, IL 61820 & Dept. Psychobiol. Univ. Calif., Irvine, CA 92717.

A previous study indicated larger layer V pyramidal neuron apical dendritic fields in dorsolateral anterior cortex opposite trained forelimbs following unilateral or bilateral training of rats to reach into a tube for food (Larson & Greenough, Soc. Neurosci. Abstr., 7, 65, 1981). In that study, the neocortical sample region was that of lowest threshold for elicitation of forelimb movement by epidural electrical stimulation, the location of which varied considerably across subjects. More recent analyses of 2-deoxyglucose uptake during reaching have indicated a very precise and consistent anterior cortical region of high activity (Fuchs et al., Soc. Neurosci. Abstr., 9, 54, 1983). In the present experiment we quantified apical dendritic branching of neurons in that region. Young adult rats were trained, over 33 50-reach sessions, to reach with the preferred forepaw (PRAC), the nonpreferred forepaw (REV), both forepaws (ALT), or nontrained (CONT). The cortical region identified in the 2DG study was projected onto Golgi sections through anterior cortex and apical dendritic branching of layer Va and Vb pyramidal cells within it was analyzed. The number of branches at each order of bifurcation from the apical dendrite, the length of terminal and bifurcating branches, and total dendritic length were analyzed. There were sublaminar differences, but no interactions of laminar position with training condition, so data from Va and Vb neurons were pooled. Results indicated more extensively branched apical dendritic fields, with longer terminal branches and more bifurcations, in hemispheres opposite trained forelimbs across all groups. Trained vs. nontrained hemisphere differences in the between subject (ALT vs. CONT) comparisons were larger than in the within subject comparisons (trained vs nontrained hemispheres of PRAC + REV), but significant effects of training were seen in both cases. Within groups, ALT and CONT showed no significant interhemispheric differences, while the trained hemispheres of REV rats had significantly more total dendritic length, more branches across all orders, and more second order branches than untrained hemispheres; those of PRAC rats had significantly more second order branches. This supports previous findings that training alters dendritic branching of adult neocortical neurons. The within subject effects of training are unlikely to arise from general hormonal or metabolic consequences of training, as opposed to events specific to the hemisphere governing movement of the trained forelimb. Supported by NSF BNS82-16916.

321.13 NEURAL SUBSTRATES OF SPONTANEOUS EXPLORATORY BEHAVIOR. L. D. Devenport and R. L. Hale* Department of Psychology, University of Oklahoma, Norman, OK 73019.

Within even the most stable terrestrial habitat, mammals may expect to experience unheralded change. Adjustment to such fluctuations is probably the basis for the small but continuous rate of spontaneous exploration that they emit without provocation. This behavior permits detection of more favorable places, times, rates, topographies, and so forth (Devenport, 1983). The neural basis of exploration of any kind--with the notable exception of novelty-induced spatial exploration (O'Keefe & Nadel, 1978)--has received little study. The present experiments examined the relative contributions of prefrontal cortex and the hippocampus to the production of spontaneous exploration along three dimensions.

Lesions were produced by aspiration or electrolysis and were intended to be complete. Rats with parietal ablations and sham operations served as controls. Exploratory activities chosen for observation were 1) temporal exploration of barpress rates in an operant chamber involving the degree of sampling of interresponse intervals; 2) route exploration within the radial arm maze indexed by the variety of successive arm choices; 3) standard instances that included rearing, visual scanning, sniffing, etc. Reward contingencies were established that neither favored or impeded the expression of exploratory responses: A reward replacement procedure was used in the maze, a random-interval schedule in the operant setting. Data were gathered from 16 days of observation.

Rats bearing hippocampal lesions exhibited very limited exploration of any sort, each category being significantly lowered to about the same extent relative to controls. Prefrontal damage yielded a single mild impairment of route investigation in the maze. The effects on exploration were not accompanied by other performance impairments as measured by such conventional indices as running time or barpress rate.

These results suggest that spontaneous exploration, along a variety of dimensions, as well as that induced by novelty (O'Keefe & Nadel, 1978), may be controlled by a limited part of the CNS.

Devenport, L. D. (1983). In Animal Cognition and Behavior, North-Holland.
O'Keefe, J. & Nadel, L. (1978). The Hippocampus as a Cognitive Map, Clarendon.

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321.14 EFFECTS OF DORSAL AND MEDIAL CORTEX LESIONS ON THE ACQUISITION AND RETENTION OF A GO-NO-GO DISCRIMINATION BY TURTLES. W. Grisham* and A. S. Powers. Bryn Mawr College, Bryn Mawr, PA 19010.

The telencephalon of turtles and other reptiles has 3 areas that are designated as being cortical; the lateral or pyriform cortex, the dorsal cortex (DC), which has been designated as a visual/general cortex, and the medial cortex (MC), which has been described as being equivalent to the hippocampus.

A deficit in the reversal of a simultaneous pattern discrimination after dorsal cortex lesions has previously been reported by this lab. It was hypothesized that the basis for this reversal deficit was an inability to withhold responses to the S-. Accordingly, DC lesions were made in animals that had already learned a go-no-go pattern discrimination ($n = 4$). It was predicted that if DC lesions resulted in an inability to withhold responses, performance on the discrimination would deteriorate post-operatively. The effects of DC lesions were compared to those of medial cortex lesions ($n = 4$) and sham lesions ($n = 3$). All animals showed nearly perfect retention post-operatively. The hypothesis was therefore rejected.

The experiment was repeated with animals that had no preoperative training. The performance of animals with DC lesions ($n = 4$) was compared to the performance of animals with MC lesions ($n = 3$) which were both compared to the preoperative data of the subjects in the previous experiment as well as added shams ($n = 2$). Animals with DC lesions required a greater number of days of training to reach criterion relative to controls ($t = 2.71$, $p < .05$). No consistent effect of MC lesions was found.

Since no retention deficit was found, the underlying basis for this acquisition deficit cannot be either a loss in motor function or a motivational loss. Also, since DC-lesioned animals showed good differential response latencies between the S+ and S- (despite the deficit), a sensory impairment seems unlikely. It is concluded that the deficit was associative and arose as a result of a retardation in the acquisition of excitatory stimulus properties by the S+. Further, this study shows that the dorsal and medial cortex are functionally different.

322 SYMPOSIUM. TOWARDS A SECOND GENERATION UNDERSTANDING OF NEURAL CIRCUITS: THE ROLE OF MODULATION. E. Marder, Brandeis University (Co-Chairperson); J. C. Weeks, UC Berkeley (Co-Chairperson); P. A. Getting, University of Iowa; R. L. Calabrese, Harvard University.

What do we need to know to understand how a neuronal circuit actually operates, either to generate behavior or process information? Previously, conventional wisdom held that it was only necessary to identify the constituent neurons of a circuit and describe their synaptic interactions and intrinsic excitability characteristics. However, in recent years it has become clear that these properties are contingent upon many variables including the presence of hormones and neuromodulators. In this symposium we will use several well-characterized invertebrate preparations to illustrate that modulation of neuronal excitability, synaptic strength, and dendritic structure (features previously assumed to be relatively invariant) enables neuronal circuits to express a variety of outputs or "states", both during development and in the mature animal. Comparison of circuits with quite different functions, from a variety of phyla (molluscs, arthropods and annelids), will allow us to articulate for the general audience the novel principles emerging from the second generation of study of small neuronal circuits. The speakers will presuppose no knowledge of the peculiarities of their preparations, but will illustrate basic concepts derived from them.

J.C. Weeks will discuss the effects of blood-borne peptide and steroid hormones on neuronal structure and motor pattern output during metamorphosis of the tobacco hornworm, Manduca sexta. P.A. Getting will discuss the hypothesis that motor networks are not "hard-wired" but that inputs can reorganize the interactions within a network and thereby alter the function of the network. R.L. Calabrese will present data that show that the neuropeptide FMRFamide is present in the leech and that it affects the period of the heartbeat central pattern generator and the myogenic properties of hearts. E. Marder will present data from the stomatogastric ganglion of decapod crustaceans that show that a large number of different neurotransmitters and neuromodulators appear to act on the same neuronal circuit so that it can produce a variety of neural outputs.

323 **SYMPOSIUM: ORIGINS OF ORIENTATION SELECTIVITY IN MAMMALIAN VISUAL CORTEX. M.P. Stryker, Univ. of Calif. San Francisco, (Chairman); P.H. Schiller, M.I.T.; J.G. Malpeli, Univ. of Illinois; A.M. Sillito, Univ. Col., Cardiff; D. Ferster, Northwestern Univ.; P.P. Heggelund, Univ. of Oslo.**

**The mechanism by which cortical neurons construct oriented receptive fields out of the non-oriented visual input from the LGN is not fully understood. Hubel & Wiesel first proposed that orientation tuning arises from convergent excitation from geniculate neurons whose receptive field centers are aligned along the axis of orientation of the postsynaptic cell. In addition, the presence of ON and OFF regions in simple cell receptive fields suggests that presynaptic geniculate cells with ON- and OFF-center receptive fields are arranged in adjacent rows. Antagonism between the rows could contribute to the attenuation of cortical responses to non-optimally oriented stimuli. Intracellular studies of synaptic potentials in simple cells favor this view, since EPSPs and IPSPs are both well oriented, always having the same preferred orientation in any one cell (FERSTER).**

**That antagonism between ON- and OFF- center inputs plays little role in orientation selectivity is suggested by the observation that suppression of ON-center cells with intraocular APB has little effect on cortical orientation selectivity (SCHILLER). Not only does excitation alone appear to provide an inadequate explanation for orientation, but evidence will be presented for a contribution by inhibitory connections. Application of GABA antagonists to cortical cells can abolish orientation selectivity (SILLITO), suggesting that inhibition between neurons with different orientation preferences is important in establishing selectivity. Alternatively, the spatial arrangement of unoriented inhibitory inputs may contribute to orientation selectivity in a manner similar to that originally suggested for excitation from the LGN (HEGGELUND).**

**Whatever the mechanism, it appears that orientation selectivity is set up independently several times within a single column; inactivation of layer 4 after cobalt injections into the LGN leaves other cortical layers functioning almost normally (MALPELI).**

## MOLECULAR BIOLOGY OF GENE EXPRESSION AND NUCLEIC ACIDS V

324.1 CLONING OF A HUMAN CHOLINE ACETYLTRANSFERASE cDNA. W. Strauss, D. Hilt, C. Puckett*, J. Kamholz*, E. Neale, P. Nelson, and M. Nirenberg, Lab. of Dev. Neurobiol., NICHD, Lab. Biochem. Gen., NHLBI, Lab. Mol. Biol., NINCDS, NIH, Bethesda, MD 20205. L. Hersh, Dept. of Biochem., U. Texas Hlth Sci. Ctr, Dallas, TX. 75235.

A cDNA clone corresponding to human brain choline acetyltransferase (ChAT) has been identified tentatively by immunologic screening of a λgt11 library prepared from human basal ganglia mRNA. Approximately $2.75X10^5$ recombinant phage plaques were assayed for the production of β-galactosidase-ChAT fusion protein that was reactive with AB865, a monospecific rabbit antibody directed against affinity purified ChAT from human placenta. Twenty-two plaques reacted with AB865 of which 8 were assayed a second time. One positive clone, λChAT7, containing an 1100 bp insert was identified. Assuming that only one-sixth of the clones in the library contain inserts in the correct orientation and reading frame for expression, the data suggest that the abundance of ChAT mRNA in the human basal ganglia is 0.006%. Lysogens of λChAT7 were made in E. coli Y1089 and grown in the presence of 10 mM IPTG to induce the synthesis of β-galactosidase fusion protein. A 160 kD protein synthesized by the λChAT7 lysogen was recognized by both anti-β-galactosidase and AB865 antibodies on western blots. Incubation of AB865 with proteins from induced lysogens of λChAT7, but not λgt11, reduced the immunohistochemical staining of cholinergic neurons. Northern blots of total cellular RNA prepared from human neuroblastoma (MCIX C) cells that synthesize ChAT demonstrated that the DNA insert excised from λChAT7, purified by agarose gel electrophoresis and labeled by nick translation hybridized to a band of RNA approximately 4200 nucleotides in length. The insert from λChAT7 hybridized with a RNA band of the same size in poly $A^+$ mRNA prepared from NS20Y cells, a clonal mouse neuroblastoma cell line with high ChAT activity, but not from N1E-115 cells, an adrenergic neuroblastoma derived from the same tumor (C1300) that lacks ChAT activity. These data suggest that the λChAT7 insert hybridizes to choline acetyltransferase mRNA. We currently are working to confirm this hypothesis by hybrid-arrested and hybrid-promoted translation.

324.2 IDENTIFICATION OF DNA COMPLEMENTARY TO DOPA-DECARBOXYLASE mRNA. V. R. Albert, and T. H. Joh, Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

Dopa-decarboxylase (DDC) is an important neurotransmitter synthetic enzyme, essential for the production of the catecholamines (dopamine, norepinephrine and epinephrine) as well as the indolamine serotonin. Despite its neuron specific function, DDC is also expressed in relatively large amounts in tissues where no monoamine neurotransmitters are synthesized. These include liver, kidney, some peptide-producing cells, and in areas of the brain and spinal cord which contain no monoamines. In order to better understand the unusual expression of this enzyme at the molecular level, a cDNA clone complementary to DDC mRNA was isolated. Because of the low abundance of this enzyme, it was necessary to employ a method whereby large numbers of recombinant clones could be screened directly. Thus a bovine adrenal cDNA library containing over $10^7$ recombinants was generated in the expression vector, lambda gt11. In this vector, cDNAs were inserted into the β-galactosidase gene, resulting in the expression of β-galactosidase fusion proteins. These fusion proteins were screened immunologically using highly specific antibodies previously raised to the protein purified from bovine adrenal medulla. Screening of 700,000 recombinants resulted in the isolation of a single positive clone. This clone, lambda DDC 46, was positively identified as a DDC clone by its ability to hybrid select mRNA coding for the 56,000 MW protein which was specifically immunoprecipitated with DDC antibody. By Northern blot analysis the 2.1 kb insert hybridizes to a bovine adrenal mRNA of approximately 2.3 kb. A similar size message can also be detected in bovine liver and kidney mRNA. RNA coding for rat DDC is slightly smaller, approximately 2.2 kb, in brain, liver and adrenal. Although preliminary immunochemical comparisons of the enzyme in various bovine tissues had suggested there might be tissue specific differences, no such differences were found at the moleular level. Furthermore, Southern blot analysis reveals that a single gene codes for this enzyme in bovine tissue. (Supported by NIH Grants, NS19002 and MH24285.)

324.3 BRAIN GLUTAMATE DECARBOXYLASE (GAD) CDNA CLONED ON THE BASIS OF ITS ANTIGENICITY AND ABILITY TO PRODUCE GABA. D.L.Kaufman*, J.F.McGinnis*, T.L.Wood* and A.J.Tobin. Dept. of Biology, Univ. of California, Los Angeles, CA 90024.

GAD converts the amino acid glutamate to GABA, the major inhibitory neurotransmitter in the mammalian central nervous system. Lesions in the GAD gene have been suggested as the cause of several neurogenetic disorders, including Huntington's disease, Parkinson's disease, and epilepsy, in which altered synthesis of GABA may cause the observed motor dysfunction.

We have used recombinant DNA techniques to isolate a cDNA clone encoding GAD from a library prepared in the bacterial expression vector gt-11. Putative GAD clones were detected by screening the library with an antiserum to purified rat GAD (Oertel et al., 1981) and purified until plates contained only immunologically positive plaques. DNA isolated from these phage contained a 2.4 kb cDNA insert. Protein analysis by immunoblotting was consistent with the production of a fusion polypeptide that reacts with antibodies to both E. coli beta-galactosidase and rat GAD. The fusion protein reacted with three independently derived anti-GAD sera: sheep anti-rat GAD, sheep anti-pig GAD, and rabbit anti-mouse GAD. These sera were graciously provided by Drs. I. Kopin, D. Martin, and J. Vaughn. In immunoblotting experiments all three anti-sera recognized two polypeptides (60 and 63 kd) in cat, human, and mouse brain extracts.

We confirmed the identity of the putative GAD clones by demonstrating enhanced GAD enzymatic activity in bacteria infected with lambda-GAD. We have partially purified the fusion proteins by gel filtration and ion-exchange chromatography. All of the GAD enzymatic activity in the purified fusion protein can be removed from solution by immunoprecipitation with anti-beta-galactosidase. The fusion protein converted uniformly-labeled $^{14}$C-glutamate to equimolar amounts of $^{14}CO_2$ and $^{14}$C-GABA.

RNA blotting analysis reveals that GAD mRNA (approximately 3.7 kb) is present in human, cat, mouse, and rat brain, but not in liver or kidney. These data are consistent with the GAD gene being transcribed in a tissue-specific manner. Our data do not reveal the presence of (1) multiple mRNA species which could give rise to the several forms of GAD which have been isolated from mammalian brain and (2) the presence of any homologous GAD-like genes transcribed in liver or kidney.

This work was supported by grants to AJT from the Dystonia Medical Research Foundation and the NIH (#NS20356 and NS22256) and to JFM (#HD05615). DLK is supported by a USPHS Training Grant in Cell and Molecular Biology (#GM07185) and TLW by a Training Grant in Integrative Biology (#GM07191).

324.4 MOLECULAR CLONING OF cDNA AND GENOMIC DNA SEGMENTS CODING THE NERVE CELL-SPECIFIC PROTEIN, SYNAPSIN I. L.J. De Gennaro and M.W. Kilimann (SPON:M.Reddington) Max Planck Institute for Psychiatry,Dept.Neurochemistry D-8033 Martinsried, West Germany

Synapsin I is a phosphoprotein associated with the neurotransmitter vesicles of presynaptic termini. It appears to be present in all neuronal cells, but not in non-neuronal tissues. Synapsin I biosynthesis is developmentally regulated and correlates with synaptogenesis. The tissue specific and developmentally regulated expression of synapsin I make it an interesting subject for a molecular genetic study. We have isolated cDNA clones from rat brain RNA coding for this protein. Synapsin I mRNA was specifically enriched to a relative abundance of ca. 10% by immunoadsorption of polysomes. Employing this enriched mRNA, a cDNA library was prepared and screened by differential colony hybridization with ss-cDNA prepared from synapsin I mRNA or total poly (A)+ RNA. Thirteen plasmids which exhibited distinctly stronger hybridization with the synapsin probe were futher characterized by restriction mapping. They all proved to be colinear. In hybridization-selection experiments several of these plasmids specifically selected synapsin I mRNA which was identified by in vitro translation and immunoprecipitation of the translation products. In Northern blot experiments, probe prepared from one of these cDNAs hybridized to two RNA species of ca. 5800 and 4500 bases in length. These putative synapsin I mRNA species were found in RNA from brain and PC12 cells, but not from liver, skeletal muscle or cardiac muscle. The cDNA clones described above have also been used as probes for the identification of synapsin I genomic segments contained in a rat genomic DNA library cloned in the EMBL-3 phage vector. These genomic segments have been analyzed by restriction mapping, R-loop analysis, and DNA base sequencing.

324.5 IDENTIFICATION OF MULTIPLE RAT STRIATAL TACHYKININ mRNA'S BY cDNA CLONING AND PRIMARY STRUCTURE ANALYSIS. J.E. Krause and J.M. Chirgwin*. Dept. Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

Substance P (SP) and substance K (α-neurokinin; SK) represent two mammalian members of the tachykinin peptide family. Whereas much basic neurobiology of substance P systems has been learned from anatomical and physiological studies performed in the rat, a biochemical and molecular biological examination of tachykinin biosynthesis and its regulation has been hindered since the primary structure of rat preprotachykinin(s) and the characteristics of its expression are not well understood. Thus, the present experiments were performed both to understand the primary structure of rat substance P precursor protein(s) from molecular cloning experiments and to provide tools for the study of the regulation of tachykinin gene expression.

Poly(A)$^+$ RNA was isolated from the rostral portion of the caudate nucleus, the site of striatonigral substance P cell bodies. A cDNA library was constructed in pUC 19 and consisted of 1.2-1.5x10$^5$ recombinants. Colony hybridization analysis for tachykinin-encoding sequences was performed by using $^{32}$P-end labeled synthetic oligonucleotides, corresponding to partial SP (a 14-mer; $T_m$=42°) and partial SK (a 20-mer; $T_m$=60°) sequences. Approximately 20 positive colonies were isolated based upon specific hybridization to SP-related (20/20) and SK-related (19/20) sequences. The results from extensive restriction enzyme mapping and cross-hybridization analyses demonstrated the relatedness of the 20 clones and were used to classify them into three subsets. The cDNA inserts from selected clones of each subset were directionally subcloned into M13 for nucleotide sequence analysis using the Sanger dideoxy method. Partial nucleotide sequence analysis confirmed the presence of near full length tachykinin encoding cDNA sequences in each subset of clones. Certain regions within the putative coding sequence of each subset of clones displayed a high degree of homology to recently described bovine preprotachykinin cDNA clones (Nawa et al., Nature 306:32, 1983), whereas other regions within the coding sequence contained additional sequence information. This, combined with the results from RNA blotting experiments using poly(A)$^+$ RNA from different CNS and peripheral tissues, suggested the presence of multiple preprotachykinin mRNA species. Currently, $S_1$ nuclease mapping experiments are being performed to quantitate the steady-state levels of each subset of preprotachykinin mRNAs in different CNS regions.

Therefore, these results demonstrate the existence of multiple mRNA species coding for the rat preprotachykinins. The cDNA's corresponding to these mRNA's should prove useful for investigating the regulation of rat tachykinin gene expression.

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324.6 STRUCTURAL ORGANIZATION OF THE HUMAN AND RAT TACHYKININS GENES. H-U Affolter*, A. C. Young* and T. I. Bonner* (SPON: V. Hook). Lab. of Cell Biology, National Institute of Mental Health, Bethesda, MD 20205.

The genes coding for the human and rat preprotachykinins RNA were isolated from genomic libraries. A rat library was screened with a synthetic oligonucleotide (72-mer) derived from the known bovine cDNA sequence. A 1 kbp fragment of the rat gene containing the first exon as well as promoter region was used to probe a human genomic library. Detailed restriction maps were established and portions of the genes sequenced and compared to the bovine gene. The genes consist of 7 exons of identical length as well as 6 introns of similar length. Two of the exons code for the tachykinins substance P (exon 3) and substance K (exon 6). The rat gene was furthermore found to contain on the coding strand and within the 6th intron a "brain ID" sequence. While the exons are highly conserved in the coding region, crosshybridization experiments and partial sequence comparison revealed no obvious sequence homologies in intronic regions. There is, however, a 200 nucleotide region of substantial homology (80%) preceding exon 1 by 40 bases. This region presumably reflects conserved regulatory sequences.

The expression of the rat gene in various regions of the brain as well as the adrenal, spinal cord and the liver was analyzed using the northern blot technique as well as RNA protection experiments. RNA hybridizing to various probes derived from the rat gene was detected in the striatum, the hypothalamus and in the adrenal. Determination of the structure of these tachykinin genes will allow for the future identification of the processes regulating the expression of substance P and K in the central nervous system as well as peripheral tissues.

324.7 THE HUMAN VASOACTIVE INTESTINAL PEPTIDE GENE: POSSIBLE REGULATION BY ALTERNATIVE SPLICING? I. Gozes[1], M. Bodner*[1,2], Y. Shani*[1], R. Avidor*[1], E. Gileadi*[1] and M. Fridkin*[2]. Departments of [1]Hormone Research and [2]Organic Chemistry, The Weizmann Institute of Science, 76100 Rehovot, Israel.

We have recently isolated the human gene coding for vasoactive intestinal peptide (VIP). This was achieved by screening a human genomic library using synthetic oligodeoxynucleotides as specific hybridization probes. Six exons were thus far discovered and sequenced. Among the RNA coding sequences are two short exons, separated by a 0.75Kb DNA stretch, one encoding VIP and the second encoding PHM-27 (a peptide having an N-terminal histidine and C-terminal methionine amide, closely related in sequence and activity to VIP). Each of these two exons encodes its respective hormone, as well as the post-translational processing signal sequences. In general, the overall structure of the VIP gene is quite similar to the structure of genes coding for the peptides, glucagon and growth-hormone-releasing-hormone. These peptides are members of a family of peptides showing similarity in their primary amino acid sequences. Thus, the similarities among these peptides are both at the peptide level, as well as at the gene level.

The 3' splice sites of the VIP and the PHM-27 encoding exons contain an identical stretch of 9 nucleotides. At the cDNA level, the 3' splice sites contain the same stretch of six nucleotides which are identically spliced. The occurrence of VIP and PHM-27 on two separate exons of the human genome and the homology of their 3' splice site may allow alternative RNA processing, yielding cells producing one peptide or the other. To study this question, we have constructed specific oligodeoxynucleotide probes to either the VIP exon or the PHM-27 exon, and used them as hybridization probes to screen various tissues for the respective mRNA. Our preliminary results suggest that differential RNA processing may well occur. To corroborate these findings, we are in the process of constructing specific RNA probes for hybridization on the one hand, and cloning the respective cDNAs on the other.

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324.8 CLONING AND CHARACTERIZATION OF MESSENGER RNA FROM RAT BRAIN THAT MAY LACK A POLY(A) TAIL. D.A. Harris, A.A. Sherbany*, and J. Brosius*. Depts. of Neurology and Human Genetics, and Center for Neurobiology & Behavior, Columbia Univ. College of Physicians & Surgeons, New York, N.Y. 10032.

There is evidence that a substantial number of mRNA molecules in rodent brain lack the poly(A) tail usually found at the 3' end of eukaryotic messengers. Moreover, many of these poly(A)$^-$ mRNAs appear to be brain-specific and to be synthesized only postnatally during brain development (Hahn, W.E., et al., Cold Spring Harbor Symp. Quant. Biol., 48:465, 1983; Chikaraishi, D.M., et al., ibid., p. 309). To document rigorously the existence and properties of this class of RNA, we have been attempting to clone and characterize these molecules using recombinant DNA techniques. The clones obtained will be useful for determining whether poly(A)$^-$ RNAs in brain are simply poly(A)$^+$ RNAs that have been artifactually truncated at their 3' ends, or whether they are distinct polysomal messengers capable of undergoing translation into protein.

Polysomal RNA from rat brain was fractionated on oligo-(dT)-cellulose, and poly(A)$^-$ RNA was tailed with bacterial poly(A) polymerase and used as a template for synthesis of cDNA. Single-stranded cDNA was annealed with a cocktail consisting of liver and kidney cytoplasmic RNA and brain poly(A)$^+$ RNA, and hybrids were removed by chromatography on hydroxyapatite. This purification step was expected to remove sequences derived from ribosomal RNA, which are major contaminants, as well as sequences that are not brain-specific or that are shared by the poly(A)$^+$ and poly(A)$^-$ RNA populations. The purified cDNA was then cloned into the plasmid pBR322.

Recombinants were screened by hybridizing bacterial colonies with a cloned ribosomal RNA probe, and by using the cDNA inserts as probes in Northern blots with brain, liver, and kidney RNA. At least six clones not derived from ribosomal RNA were identified, four of which have been sequenced. Several of these cDNA candidates fail to hybridize with a corresponding RNA in Northern blots of either poly(A)$^-$ or poly(A)$^+$ RNA from brain; this result is obtained when the cDNAs are used either as nick-translated probes or as single-stranded probes of higher specific radioactivity cloned in phage M13. We therefore estimate that these clones are derived from low-abundance RNAs. We are currently employing affinity chromatography with ribosomal DNA-Sephacryl to deplete poly(A)$^-$ RNA preparations of ribosomal RNA, and thereby enrich for messenger sequences; we expect that this purified RNA will permit more sensitive detection of poly(A)$^-$ species. Although scarcely represented at the RNA level, the sequences that we have identified are readily detectable by Southern hybridization in genomic DNA, where they appear to be derived in most cases from single-copy genes.

At present, it is not possible to determine definitively whether the clones we have identified represent a class of brain RNA that is structurally distinct from poly(A)$^+$ RNA. Our results indicate, however, that if such species exist, then at least some of them are present only at low levels in total brain RNA.

324.9 CLONING OF NONADENYLATED POLYSOMAL RNA FROM RODENT BRAIN. B.J. Snider* and M.R. Morrison. Depts. of Biochemistry and Neurology, Southwestern Graduate School, UTHSCD, Dallas, Texas, 75235.

RNA complexity measurements have revealed the presence of a large complex population of nonadenylated polysomal RNAs in rodent brain that are distinct from the polyadenylated (Poly(A)$^+$) RNA population (Chikaraishi, D.M., Biochemistry 18:3249, 1979, and Hahn, W.D., Cell 18:1341, 1979). Recently, two representatives of this class have been identified in a rat genomic library screened for rare brain RNA transcripts (Chikaraishi, D.M., Cell. and Mol. Biology, 4:2187). In order to further characterize this RNA population, we are constructing a cDNA library representing nonadenylated polysomal RNAs from rat brain. Because even small levels of nuclear RNA in cytosolic preparations could artefactually elevate observed levels of cytoplasmic RNA complexity, we used a cloned probe homologous to the abundant small nuclear RNA U1 (provided by T. Manser and R.F. Gesteland, University of Utah) to quantify nuclear contamination. Homogenization of brains in hexylene glycol produced a cytoplasmic fraction with significantly less nuclear contamination than homogenization in buffers containing sucrose and Triton X-100. Whole rat brains are homogenized in hexylene glycol and polysomes are prepared by centrifugation over a sucrose cushion. RNA is isolated from the polysomal fraction by treatment with Proteinase K followed by phenol extraction and ethanol precipitation. Poly(A)$^+$ and poly(A)$^-$ RNAs are separated by two passages over oligo(dT) cellulose. Poly(A)$^+$ RNAs are prepared for cloning by adding a poly(A) tail to their 3' ends with E.Coli Poly(A) Polymerase. After the addition of approximately 80-100 adenylate residues per RNA, the first cDNA strand is synthesized using Reverse Transcriptase (RT) and an oligo(dT) primer. Since the RNA population contained roughly 98% ribosomal RNAs (rRNA), cDNAs representing rare nonribosomal RNAs are selected for further cloning by a subtraction hybridization procedure. After synthesis of the first cDNA strand, the RNA template is hydrolyzed and the cDNA is annealed to an excess of brain polysomal nonadenylated RNA (the template RNA) to a Rot of 1000 and then to an excess of nonadenylated RNA prepared from liver polysomes to a Rot of 5000. The single-stranded cDNA (10-20% of starting cDNA) is recovered by HAP chromatography. This cDNA is A-tailed with terminal deoxynucleotidyl transferase and a second strand synthesized with AMV RT and an oligo(dT) primer. The double-stranded cDNAs will be cloned into M13mp8 and λgt10 and these libraries will be screened for clones representing rare nonadenylated RNAs by using several positive and negative screens. Analysis of clones generated by this method should provide clues to the role of nonadenylated RNAs in brain development and function.

324.10 GENES REGULATED BY NERVE GROWTH FACTOR IN PC12 CELLS. D.G.B. Leonard*, L.A. Greene* and E.B. Ziff* (SPON: R. I. Frank). Depts. of Biochemistry and Pharmacology, New York Univ. Med. Ctr., New York, NY 10016.

PC12 cells are a rat pheochromocytoma-derived cell line which has a chromaffin-like phenotype in cell culture. When treated with nerve growth factor (NGF), the cells acquire a sympathetic neuron-like phenotype, characterized by the cessation of proliferation, the extension of neurites and the acquisition of electrical excitability. In order to identify genes that may be important to the characteristics of differentiated sympathetic neurons, cDNA libraries made from both NGF-treated and -untreated PC12 cells were differentially screened. cDNA was made from polyA$^+$ RNA and inserted into the lambda gt10 vector (Huynh, T.V., R.A. Young and R.W. Davis in DNA Cloning Techniques: A Practical Approach. D. Glover, ed. IRL Press, Oxford. In Press). Both libraries contain greater than 1 million recombinant clones. Each library was screened with single-stranded $^{32}$P-labeled cDNA prepared from three different cell types: 1) untreated PC12 cells; 2) PC12 cells treated with 50 ng/ml NGF for 2 weeks; and 3) a spontaneously arising mutant cell line (F4) which was clonally-derived from PC12 cells and which lacks a number of differentiated PC12 properties including catecholamine synthesis and the capacity to bind or respond to NGF. Clones with different autoradiographic signals between the three cDNA types were picked and rescreened. At the present time, Northern analysis has confirmed that 6 clones represent mRNAs which are quantitatively different in the three cell types. One clone specifically hybridizes to a mRNA of approximately 820 nucleotides which is more abundant in the NGF-treated PC12 cells than in the untreated cells and is relatively rare in the F4 cells. In contrast, another cDNA clone appears to recognize two mRNA species of approximately 4600 and 1400 nucleotides which are abundant in the untreated PC12 cells, present at lower levels in the NGF-treated PC12 cells and undetectable in the F4 cells. Three additional clones were identified which hybridize with mRNAs that are present in equal amounts in NGF-treated and -untreated PC12 cells, but are either undetectable or much less abundant in the F4 cells. Further characterization of these and other clones will be discussed.

This investigation was supported by NIH Grants NS16036 and GM-30760, and by ACS Grant MV-75B. DGBL was supported by Training Grant 5T32 GM07308-10 from the National Institute of General Medical Sciences.

324.11 NERVE GROWTH FACTOR RAPIDLY INDUCES ORNITHINE DECARBOXYLASE mRNA IN PC12 PHEOCHROMOCYTOMA CELLS. S. Feinstein* (1), Sharon Dana* (2), Lisa McConlogue* (2), Philip Coffino* (2) and Eric M Shooter (1). (1) Dept. of Neurobiology, Stanford Univ. Sch. Med., Stanford, CA 94305 and (2) Dept. of Microbiology, Univ. of California, San Francisco, CA 94143.

The mechanism by which nerve growth factor (NGF) stimulates ornithine decarboxylase activity (OrnDCase; EC.1.1.17) in the rat pheochromocytoma cell line PC12 was investigated. As previously demonstrated, NGF rapidly induces OrnDCase activity in a dose dependent manner, with maximal enzymatic activity at 4 - 6 hours after exposure to NGF. Activity subsequently returns to near basal levels. A cloned OrnDCase cDNA was used to analyze the levels of OrnDCase RNA. In response to NGF administration, OrnDCase RNA levels are induced. The time course of the OrnDCase induction paralleled that of the enzyme activity induction, and the magnitude of both inductions was quantitatively the same. Increased concentration of OrnDCase RNA is clearly detected at the earliest time point examined, 2 hours. No change was observed in the size of OrnDCase RNA. The dose response curves for both OrnDCase RNA and enzymatic activity inductions were also similar. Thus, increased OrnDCase RNA levels fully account for, and are responsible for, the induction of activity. Further, one-third of the OrnDCase RNA induction is unaffected by cycloheximide treatment, but fully blocked by actinomycin D treatment, suggesting that NGF acts through at least two mechanisms to mediate the OrnDCase induction. The first mechanism is cycloheximide insensitive and the second mediated through an event requiring ongoing protein synthesis. Both mechanisms require ongoing transcription, as evidenced by the complete sensitivity of the induction process to actinomycin D.

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324.12 **EXPRESSION OF THE EGG LAYING HORMONE GENE FAMILY IN THE HEAD GANGLIA OF APLYSIA CALIFORNICA.** M. SHYAMALA*., J.R. NAMBU* AND R.H. SCHELLER. Department of Biological Sciences, Stanford University, Stanford, CA.94305

The egg laying hormone (ELH), which is encoded in a small multigene family, promotes release of eggs from the ovotestis and also modifies the activity of neurones in the abdominal and head ganglia. A member of the gene family expressed in the bag cells encodes ELH and a distinct subset of the family expressed in the atrial gland encodes the peptides A and B. In situ hybridization studies using radiolabelled cloned DNA probes have indicated that the ELH gene family is expressed in neurones of the abdominal, buccal, cerebral, and pleural ganglia in addition to the bag cells and the atrial gland. Immunohistochemical studies using anti-ELH antibody have revealed a network of cells producing ELH immunoreactive proteins in all of the major ganglia of the nervous system except the pedal ganglia. In order to study the nature of the ELH gene family transcripts expressed in the various ganglia, a radiolabelled cDNA probe was employed to screen a cDNA library constructed from ring ganglia (cerebral, pedal, and pleural ganglia) poly A(+) RNA. Several clones that are homologous to the ELH cDNA probe were isolated and characterized. Restriction endonuclease mapping and blotting of ten clones indicated that one of the clones represents an A and/or B peptide gene transcript and the other clones represent transcripts from the ELH gene. A further analysis ot two ot the clones by DNA sequencing confirmed the presence of A peptide and ELH gene transcripts in the ring ganglia. This data was strengthened by Northern blotting which indicated the presence of A and/or B peptide related transcripts in cerebral and pedal ganglia and ELH related transcripts in all of the head ganglia.

## NEUROTRANSMITTERS AND RECEPTORS IN DISEASE: MPTP MODEL OF PARKINSON'S DISEASE

325.1 AUTORADIOGRAPHIC DISTRIBUTIONS OF NEUROTRANSMITTER RECEPTORS OF HUMAN BRAINS WITH PARKINSON'S DISEASE AND PRIMATE MODELS OF MPTP-INDUCED PARKINSONISM. S. Kito, R. Miyoshi*, K. Mizuno*, H. Matsubayashi* , K. Nitta* and Y. Yamamura. Third Department of Internal Medicine, Hiroshima University School of Medicine, Hiroshima 734,Japan.

Distributions of neurotransmitter receptors in human brains of two parkinsonian patients were autoradiographically studied with use of various radioactive ligands such as $^{3}$H-lisuride (a dopamine $D_2$ receptor agonist), $^{3}$H-spiperone (a dopamine receptor antagonist), $^{3}$H-QNB (a muscarinic acetycholine receptor antagonist) and $^{3}$H-substance P, and these results were compared with morphological observations through conventional neuropathological techniques. The method of in vitro autoradiography used in this study was the one originally established by Kuhar et al. Cryostat sections of autopsied human brains were prepared and scatchard plots of saturation analysis were obtained by the same way as binding experiments with use of brain homogenate. These preliminary experiments are needed to decide optimal conditions for autoradiography. After obtaining scatchard plots, the authors proceeded to autoradiographic techniques in which incubated sections were apposed to a sheet of $^{3}$H-sensitive film and exposed for several weeks. The observed autoradiograms were computer-analysed by IBAS II of Zeiss company. Through these studies it was found that $^{3}$H-lisuride known as one of anti-parkinsonian ergot alkaloids specifically bound not only to dopamine receptors, but also to serotonergic and alpha$_2$-adrenergic receptors. In the parkinsonian brain, the density of specific $^{3}$H-lisuride binding sites was increased in the striatum, globus pallidus, cingulate gyrus, while it was decreased in the cerebellar cortex, parahippocampal gyrus, pre- and postcentral gyri. Specific binding sites of muscarinic acetylcholine receptor were increased in the putamen. Results of conventional neuropathological studies were consistent with pathology of Parkinson's disease with remarkable neuronal loss in the substantia nigra, otherwise no notable changes. Changes of $^{3}$H-lisuride binding sites in the cerebellum, parahippocampal gyrus and other cerebral cortices were regarded to represent adrenergic and serotonergic receptors.

Autoradiographic studies on three Macaca fuscatus models of MPTP-induced parkinsonism using $^{3}$H-QNB and $^{3}$H-spiperone were done likewise and the results were disscussed in comparison with human parkinsonian brains and control monkeys. In these monkey models, there was an increase of $^{3}$H-QNB binding sites in the putamen, which had been observed also in human brains. Muscarinic acetylcholine receptors were somewhat decreased in the hippocampus.

325.2 EFFECT OF CHRONIC LEVODOPA OR BROMOCRIPTINE TREATMENT ON DYSKINESIA AND [$^{3}$H]SPIPERONE BINDING IN MPTP LESIONED PARKINSONIAN MONKEYS. P. Falardeau*[1], R. Boucher[2], P. Bédard[2] and T. Di Paolo[1] (SPON: A. Dupont), [1]School of Pharmacy, Laval Univ., Quebec G1K 7P4 and Dept. of Molecular Endocrinology, Laval Univ. Hosp. Center, Quebec G1V 4G2; [2]Neurobiology Lab., Dept. of Anatomy, Laval Univ., Quebec G1J 1Z4, Canada.

Dyskinesia is one of the most frequent side effects of L-DOPA treatment in Parkinsonian patients while bromocriptine has been reported to induce little or no dyskinesia. DOPA-induced dyskinesia generally appear after long term treatment suggesting that the drug plays a role in the genesis of these movements. Moreover, they often appear on the side first affected by the disease implying that the extent of the nigral lesion and the resulting supersensitivity to dopamine (DA) may be important. The aim of this study was to compare in monkeys with a parkinsonian syndrome induced by 1-methyl-4-phenyl-1,2,5,6-tetrahydroxypyridine (MPTP) the effect of treatment with L-DOPA/carbidopa (Sinemet (R)) or bromocriptine to see if some animals develop dyskinesia and if this could be correlated with differences in post-synaptic striatal DA receptors. Five ovariectomized macacca facicularis monkeys developed a severe parkinsonian syndrome after intraveneous administration of the toxin MPTP. Injections were repeated every third day until the animals appeared bradykinetic. This was generally apparent two days after the injection. Then, one monkey remained untreated while two animals were treated daily for five months with L-DOPA/Carbidopa (100/25 mg increased after a month to 150/37.5 mg) and two with bromocriptine orally (10 mg increased after a month to 15 mg and after 2 months to 20 mg). Both drugs relieved the parkinsonian symptoms but the animals on Sinemet developed after two weeks prominent lingual dyskinesia which remained visible after each dose until the end of the experiment. In the two animals on bromocriptine no dyskinesia were observed. After sacrifice, the levels of DA, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) and [$^{3}$H]spiperone binding were studied bilaterally in the anterior and posterior caudate nucleus, anterior and posterior putamen, and in the nucleus accumbens. The loss of DA, DOPAC and HVA were equivalent in the Sinemet and the bromocriptine treated animals (> 90%) and there was a complete disappearance of the substantia nigra pars compacta. In all structures studied, the Bmax for [$^{3}$H]spiperone binding was on average 10% higher in the Sinemet than in the bromocriptine treated animals while the affinity ($K_D$) was unchanged. We therefore believe that L-DOPA and bromocriptine affect denervated postsynaptic DA receptors differently, that bromocriptine is less likely to induce agonist supersensitivity and that this probably explains the lesser tendency to induce dyskinesia after chronic treatment.

Supported by the Parkinson Foundation of Canada.

325.3 LONG-TERM EFFECTS OF MPTP ON BRAIN AND PERIPHERAL CATECHOLAMINE AND INDOLEAMINE CONCENTRATIONS OF MONKEYS. P. Bédard[1], R. Boucher[1], P. Falardeau*[2] and T. DiPaolo[2], [1]Neurobiology Lab., Dept. of Anatomy, Laval Univ., Québec, G1J 1Z4, [2]School of Pharmacy, Laval Univ., Québec, G1K 7P4 and Dept. of Mol. Endocrinology, Laval Univ. Hosp. Center, Québec, G1V 4G2, CANADA.

5 Maccaca Fascicularis monkeys developed a severe parkinsonian syndrome in the days following intravenous administration of the toxin MPTP. These animals were then divided in three groups. One monkey remained untreated while two animals were treated daily for five months with supramaximal doses of Sinemet (R) and two with bromocriptine orally. Both drugs relieved the parkinsonian symptoms. These 5 animals plus one normal monkey were sacrificed 3 days after their last drug treatment. Plasma prolactin concentrations were elevated in the untreated MPTP monkey compared to values from an intact control monkey. MPTP caused a rapid decrease of homovanillic acid (HVA) concentrations in the CSF of these monkeys within days of the toxin injections and this remained until sacrifice of the animals 5 months later. By contrast, CSF 5-hydroxyindoleacetic acid (5-HIAA) concentrations are elevated a few days after MPTP and these values return to control after 5 months. Five months after MPTP, epinephrine (E) and dopamine concentrations in the adrenal medulla of the monkeys are decreased while norepinephrine (NE) concentrations are unchanged. By contrast, NE is increased in the adrenal cortex of the MPTP monkeys while E and DA are unchanged. Catecholamines and indoleamines were assayed in the caudate, putamen, nucleus accumbens, amygdala and frontal cortex of these monkeys. In general MPTP treatment increased 5-hydroxytryptophan (5-HTP), 5-hydroxytryptamine (5-HT) and 5-HIAA concentrations in the brain regions analyzed and L-DOPA or bromocriptine treatment corrected these changes. Similar results are obtained for NE concentrations in the caudate and putamen while no effect of MPTP was observed in the accumbens and a decrease was observed in the frontal cortex. Significant changes of E concentrations after MPTP were observed only in the n. accumbens where a decrease was observed. Dopamine and its metabolites dihydroxyphenylacetic acid (DOPAC) and HVA were significantly reduced in the caudate, putamen, n. accumbens and frontal cortex. Our results show that MPTP in the long-term not only affects the caudate-putamen dopaminergic system but also dopaminergic noradrenergic, adrenergic and serotonergic systems in the brain and in the periphery. Supported by the Parkinson Foundation of Canada.

325.4 RETINAL CATECHOLAMINE METABOLISM FOLLOWING MPTP TREATMENT. *C.G. Wong. Dept. Ophthalmology, Doheny Eye Foundation Bldg., USC School of Medicine, 1355 San Pablo St., Los Angeles, CA 90033.

Recently 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has been shown to cause a syndrome similar to Parkinsonism in both man and monkeys by selective destruction of dopaminergic cells in the substantia nigra. The rabbit retina is a uniquely relevant model for studying the actions of MPTP on dopamine (DA) metabolism since other neurotransmitters such as NE or 5-HT and resulting complex interactions are not present. In this study MPTP is shown to affect DA metabolism in the pigmented rabbit retina. Rabbits were injected daily intramuscularly (IM) for 14 days at either 0.1 mg/kg or 2 mg/kg and sacrificed either 24 hrs. or 14 days after the last injection. In a second study, rabbits were injected IM with either 4 mg/kg or 2 mg/kg for 6 days daily and then sacrificed at 3, 10, and 17 days after the last injection. In a third study, four groups of rabbits were injected IM once with one of the following doses of MPTP: 0.1, 0.5, 1.0, and 2.0 mg/kg. The rabbits were then sacrificed either 24 hrs. or 16 days later. Both intravenous and intraocular injections were done also.

Overall, DA levels after a single injection of MPTP were depressed over the observed time period while that of chronically treated animals were elevated. DOPA levels were markedly elevated in all chronically treated animals except for the first time point at the higher dose of 4 mg/kg. Thus, after a single IM injection of 2 mg/kg 16 days later both DOPA and DA levels were depressed 42% and 57% of control, respectively. However, with one injection of 1 mg/kg, at both 24 hrs. and 16 days later, DOPA levels were elevated 233% and 396%, respectively, while DA levels were depressed 40% and 67%, respectively. With 0.5 mg/kg after 24 hrs., both DOPA and DA levels were depressed to 23% and 66% of normal while after 16 days, DOPA had returned to normal with DA still depressed at 83% of control. DOPA levels of animals treated chronically IM with 2 mg/kg were elevated 3, 10, and 17 days by 158%, 140%, and 132%; DA levels were within normal range after 3 days while elevated at 10 and 17 days by 353% and 156%, respectively. DOPA levels of animals treated chronically IM with 4 mg/kg were depressed 53% after 3 days while elevated 246% and 169% both 10 and 17 days after the last injection; DA levels were not significantly different after 3 days while elevated both 10 and 17 days later by 139% and 129%, respectively.

These results clearly demonstrate the dramatic changes in retinal DA metabolism following MPTP treatment by various routes. Whether the mechanisms of MPTP toxicity in the mammalian retina is similar to its selective toxicity on dopaminergic cells in the primate CNS remains to be shown. The possibility is raised, however, that the rabbit retina may provide a useful model system for studying MPTP toxicity.

325.5 EVALUATION OF CILADOPA HYDROCHLORIDE AS A POTENTIAL ANTIPARKINSON AGENT. W.C. Koller, J.Z. Fields, R. Rybicki*, J. Gordon and M. Perlow. Hines-Loyola Medical Center, Chicago Medical School, University of Illinois, Chicago, IL 60141

Ciladopa HCL (S (-)[4-(2-hydroxy-2-3',4'-dimethoxyphenyl 1) ethyl}-1-piprazinyl]-2,4,6-cycloheptatriene-1-one) is a pharmacologically active enantiomer of a troponylpiperazine derivative. Initial studies (Ayerest Laboratories) indicate that this compound enhances dopaminergic activity. Ciladopa may be a novel dopamine agonist as it is not an ergot derivative. Thus the drug may represent a new pharmacologic approach for the treatment of Parkinson's disease. We have therefore investigated ciladopa in animal models of central dopaminergic activity.

Ciladopa in rats induced dose-dependent (2.5 to 20 mg/kg) behavioral changes consisting of locomotion, head bobbing, and sniffing. Doses greater than 20 mg/kg caused toxicity. Behaviors such as rearing, grooming, and gnawing observed after apomorphine (0.25 and 0.5 mg/kg) did not occur with ciladopa. Haloperidol pretreatment (doses greater than 0.05 mg/kg) blocked ciladopa's effect. Sulpiride pretreatment (10 to 40 mg/kg) caused partial blockage. In guinea pigs ciladopa (2.5 to 20 mg/kg) produce a dose-dependent increase in locomotion and gnawing but at a lower intensity than apomorphine (0.4 and 0.8 mg/kg). Higher doses of ciladopa (40 and 80 mg/kg) caused less of a behavioral effect than lower doses suggesting partial agonism. Haloperidol pretreatment (doses greater than 0.10 mg/kg) blocked ciladopa's effect whereas sulpiride pretreatment (10 to 40 mg/kg) had no effect. Ciladopa's action either in rats or guinea pigs was unaltered by pretreatment with reserpine (2.5 mg/kg, 16 hrs. prior) or with alpha-methyl-para-tyrosine (200 mg/kg, 2 hrs. prior), indicating direct agonism. In rats with unilateral 6-OH dopamine lesions ciladopa caused contralateral turning (2.5 and 5.0 mg/kg), however, in some animals ipsilateral turning (similar to amphetamine) preceded contraversive turning. In dogs ciladopa (0.1 to 1.0 mg/kg) caused dose-dependent emesis. Rectal temperature in mice was reduced in dose-dependent fashion (2.5 to 10 mg/kg). Ciladopa at low doses (1.0 to 2.5 mg/kg) decreased locomotion suggesting activation of pre-synaptic dopamine autoreceptors.

Ciladopa is a dopamine agonist with a novel pharmacologic profile that is potentially useful for the treatment of Parkinson's disease.

325.6 REPEATED ADMINISTRATION OF L-DOPA PLUS BENSERAZIDE IN 6-OHDA LESIONED RATS: BEHAVIORAL AND BIOCHEMICAL SIGNS OF DOPAMINE RECEPTOR SUPERSENSITIVITY. C. Rouillard*, P.J. Bédard, P. Falardeau*, T. DiPaolo (SPON: R. BOUCHER). Lab. Neurobiology, Dept. Anatomy and Dept. Molecular Endocrinology, Univ. Laval, Québec, Canada, G1K 7P4.

Treatment with L-DOPA plus a peripheral inhibitor of Dopa decarboxylase is an effective treatment of Parkinson's disease. But a majority of these patients develop dyskinetic movements suggesting some type of supersensitivity related to the treatment but facilitated by denervation. We investigated behaviorally (circling) and biochemically ($^3$H-Spiroperidol binding) the effect of repeated administration of L-DOPA plus benserazide in rats bearing a unilateral lesion of the nigrostriatal pathway performed with 6-OHDA. The animals were divided in three groups; the first group receiving 8 injections of L-DOPA (100 mg/kg i.p.) plus benserazide (50 mg/kg i.p. ½ hour before) separated by 48 hours while the second group received only the first and the last injections and the third served as control and received no injection. Circling was monitored during five hours after each injection. Seventy-two hours after the last dose, the animals were sacrificed by decapitation and the striata rapidly removed for subsequent binding studies. The two groups of rats having received L-DOPA showed a significant increase of the circling response (109% and 39% respectively for L-DOPAx8 and L-DOPAx2). This increase was mostly in the first three hours while the duration of the response seemed to be unchanged. In the L-DOPAx2 group, we saw a small, non significant increase of the Bmax on the lesioned side compared to the same side of the control group. However, in the L-DOPAx8 group, the Bmax was increased by 22% ($p < 0,05$) on the lesioned side while it was decreased by 18% (n.s.) on the intact side. These results suggest that the increased behavioral response may be explained by DA receptor supersensitivity on the lesioned side combined with an increased difference in sensitivity between sides. The present results also indicate that the action of L-DOPA is different from that of Bromocriptine which under similar conditions mostly desensitizes the intact side and has little or no effect on the lesioned side (Rouillard et al., Neurosci., 10: 237, 1984). This may explain the difference in incidence of dyskinesia seen in Parkinsonian patients treated with these two drugs. (Supported by MRC and Parkinson Foundation of Canada).

325.7 CHOLINERGIC AND SOMATOSTATINERGIC RECEPTOR ALTERATIONS IN SENILE DEMENTIA OF THE ALZHEIMER'S TYPE (SDAT) AND IN ITS ANIMAL MODEL. K. Gulya[1]* T.W. Vickroy[1], M. Watson[1], W.R. Roeske[1], E. Perry[2]*, R. Perry[2]*, S.P. Duckles[1] and H.I. Yamamura[1].[1]Depts. of Pharmacology, Univ. of Arizona, Tucson, and [2]Dept. of Neuropathology, Newcastle General Hospital, Newcastle upon Tyne, England.

Biochemical and receptor binding experiments were carried out to study the cholinergic and somatostatinergic transmitter systems in post-mortem human brain samples from SDAT and age-matched patients. Alterations of the cholinergic system after ibotenic acid-induced lesions of the nucleus basalis magnocellularis (nBM) of rat were also studied. Presynaptic cholinergic markers (choline acetyltransferase,CAT; sodium dependent binding of the choline uptake inhibitor [$^3$H]hemicholinium-3, [$^3$H]HC-3) and muscarinic receptor radioligands such as [$^3$H]-*cis*-methyldioxolane ([$^3$H](+)CD), [$^3$H]pirenzepine ([$^3$H]PZ) and [$^3$H](-)quinuclidinyl benzilate ([$^3$H](-)QNB were used to detect changes in the central cholinergic nervous system of SDAT patients compared with age-matched controls. Similar comparisons were carried out in rats with ibotenic acid-induced lesions of nBM (animal model for SDAT) compared with control animals. Somatostatin receptor binding using the iodinated nonhydrolyzable dicarba analog [$^{125}$I]CGP 23,996 was also studied. These measurements were carried out in the hippocampal gyrus and cingulate and parietal cortices in humans and in the anterior cerebral cortex in rats. The results are:

| Marker | SDAT | | | Rat |
|---|---|---|---|---|
| | hippoc. gyrus | cingulate cortex | parietal cortex | anterior cerebral cortex |
| CAT | 48 * | 36 * | 31 * | 59 * |
| [$^3$H]HC-3 | n.d. | n.d. | n.d. | 59 * |
| [$^3$H](+)CD | 133 | 109 | 107 | 79 |
| [$^3$H]PZ | 75 | 116 | 77 | 90 |
| [$^3$H](-)QNB | 83 | 106 | 104 | 89 |
| [$^{125}$I]CGP 23,996 | 48 * | 47 * | 46 * | n.m. |

Significantly *$p < 0.05$ by Student's t-test. Data are shown as percent of control values. n.d. = not detected, n.m. = not measured.

We demonstrated a significant decrease in [$^3$H]HC-3 binding to ibotenic acid-lesioned rat cerebral cortices and somatostatin receptor binding in all regions from SDAT samples. CAT activity was significantly reduced in SDAT tissue as well as cortical tissue from the ibotenic acid-lesioned animals. There is no muscarinic receptor loss in SDAT or in its animal model. The selective loss of putative presynaptic cholinergic markers and the reduction of somatostatin receptor binding indicate the co-involvement of these neurotransmitter systems in the neuropathology of SDAT. Supported by NIMH Grants.

325.8 NEUROPEPTIDE Y IMMUNOREACTIVITY IS REDUCED IN ALZHEIMER'S DISEASE CEREBRAL CORTEX. M. Flint Beal, Michael F. Mazurek*, Geetinder Chattha*, Edward D. Bird and Joseph B. Martin. Department of Neurology, Massachusetts General Hospital, Boston, MA 02114.

Neuropeptide Y is a 36 amino acid peptide which has been found in high concentrations in cerebral cortex and is contained in cortical neurons. We measured concentrations of this peptide in cerebral cortex using a sensitive and specific radioimmunoassay. Postmortem brain tissue from 12 patients with Alzheimer's disease (AD) and 13 neurologically normal controls was dissected and extracted in boiling 2N acetic acid. The diagnosis of AD was confirmed neuropathologically in all cases. Neuropathologic examination in controls was normal. Post-mortem intervals were 11.5 $\pm$ 1.6 hrs in controls and 11.3 $\pm$ 2.0 hrs in AD brains.

Antibody to neuropeptide Y conjugated to bovine serum albumin was raised in rabbits. Neuropeptide Y was iodinated using the chloramine T reaction and purified using HPLC. The radioimmunoassay showed no significant cross-reactivity with other neuropeptides tested. The assay sensitivity is approximately 8 pg/tube. Twenty one cortical regions were examined. Significant ($p<.05$) reductions in immunoreactivity were seen in the following Brodmann areas: A4, 68%; A6, 58%; A12, 56%; A17, 76%; A20, 64%; A21, 54%; A22, 66%; A24, 61%; A32, 67%; A45, 49%; and A47, 52%. Smaller reductions which were not significant were seen in Brodmann areas: 8,9,10,11,25,28,38,44,46 and 3-1-2. HPLC showed that more than 90% of neuropeptide Y immunoreactivity co-migrated with standards in both Alzheimer's and control brains. Smaller peaks were seen at the void volume which may represent higher molecular weight species.

Somatostatin neurons have previously been shown to degenerate in AD cerebral cortex. These studies demonstrate loss of a second neurochemical marker of cortical neurons in Alzheimer's disease. The regions particularly affected include temporal lobe, frontal lobe and occipital cortex. Since neuropeptide Y is co-localized with somatostatin in a considerable proportion of cortical neurons the loss of immunoreactivity may in part reflect degeneration of these neurons.

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325.9 Nicotine Receptors in Human Frontal and Infratemporal Cortex: Comparison between Alzheimer's Disease and the Normal. D.D. Flynn and D.C. Mash, University of Miami School of Medicine, Miami, FL and Harvard Medical School, Boston, MA.

Reductions in the presynaptic markers, choline acetyltransferase and M2 muscarine receptors (Mash et al., 1985), have been attributed to degeneration of neurons located in the basal forebrain. Despite widespread reductions in these markers, postsynaptic M1 receptors remain unchanged in Alzheimer's disease (AD). The possibility that nicotinic-cholinergic transmission is impaired in AD brains has not been previously examined. The present study was undertaken to establish assay conditions for nicotine receptors in human postmortem cortical samples in order to determine the status of these sites in AD.

Cortical samples obtained 2-12 hours postmortem, were homogenized and assayed in Tris Ca++ buffer at pH 7.4. Membranes were incubated with (-) 3H-nicotine (7.5-30 nM) in a total volume of 1 ml for 30 minutes at 25° C. Nonspecific binding was determined by the addition of an excess of (-) nicotine and was generally less than 10% of the total binding. Membranes were collected after dilution with buffer at 4° C on filters presoaked in 0.1% polyethyleneimine. (-) 3H-nicotine binding was enhanced by the addition of Ca++ and abolished in the presence of EDTA. Association and dissociation were rapid at 25° C with $t_{½}$ values of 3 min and 5 min., respectively. Scatchard analysis of (-) 3H-nicotine binding revealed an apparent single class of binding sites with a dissociation constant, Kd, of 7 nM in agreement with the previously reported value in the rat brain (Clarke et. al., 1984). There was no effect of postmortem interval on the number of 3H-nicotine sites assayed in rat frontoparietal cortex.

In the human frontal cortex the number of binding sites, $B_{max}$, decreased with normal aging (27-85 yrs) from 2.0 pmol/g to 0.8 pmol/g. The receptor affinity and concentration in AD frontal cortex (Brodmann 10) were comparable to that in age-matched controls. In AD infratemporal cortex (Brodmann 38), the number of sites was significantly decreased by 50% ($p < 0.001$). The diagnosis of AD was confirmed neuropathologically and choline acetyltransferase activity was decreased (50-80%) in both cortical areas.

These results demonstrate that postsynaptic nicotine sites are spared in the frontal cortex in AD. In the infratemporal cortex, significant numbers of nicotine receptors remain, despite severe reductions in choline acetyltransferase activity. Further studies of the distribution and status of these sites in AD may provide a rationale for using nicotinic agonists to improve some of the cognitive deficits associated with AD. Supported by NS 19065 and NS 09211.

325.10 CALCIUM CHANNEL ANTAGONIST BINDING SITES IN FRESH HUMAN BRAIN CORTEX. F. Lafaille*, N.P.V. Nair, S. Lal*, A. Olivier*, J. Strattford*, R.M. Ford* and R. Quirion. Douglas Hospital Research Centre, Verdun, Quebec H4H 1R3 Canada, Montreal Neurological Institute and Montreal General Hospital, Montreal, Quebec Canada.

Recent data have demonstrated the presence of high affinity binding sites for calcium channel antagonists in the brain. Interestingly, these sites are mainly located in areas enriched in synaptic contacts. We have now characterized calcium channel antagonist binding sites in fresh human brain cortex using [$^3$H]PN-200-110, a highly potent dihydropyridine derivative. Fresh human brain cortex is obtained from patients undergoing surgery for removal of tumors (n = 7) or epileptogenic focus (n = 8). Only apparently normal surrounding tissues are used for assays. Samples are kept on ice before homogenization in Krebs Ringer buffer using a Brinkmann polytron at setting 5 for 17 sec. (mean delay of 52 min between removal of tissues and processing). Homogenates are then centrifuged three times for 15 min at 39,000 g and the final pellet is resuspended in Krebs buffer to yield a protein concentration of 2-2.5 mg/ml. 200 µl of this preparation is then incubated for 60 min in Krebs buffer at 37°C with various concentrations of [$^3$H]PN-200-110 (0.03 - 5.0 nM; 84 Ci/mmol, New England Nuclear) in presence and absence of 10 µM nimodipine to assess specific binding. Incubations are terminated by rapid filtration through GF/B filters followed by two 4 ml washes using cold Krebs buffer. Under these conditions, [$^3$H]PN-200-110 binds to a single class of high affinity sites is fresh human brain cortex. $K_d$ values ranged between 0.15 - 0.57 nM without apparent differences between samples obtained from tumor or epileptogenic tissues. The densities of sites ($B_{max}$) are also independent of the disease state but varied between cortical regions. The highest density is found in the temporal lobe (182.3 fmol/mg protein) followed by the fronto-temporal (180), frontal (180), fronto-parietal (177), temporo-parietal (110) and parietal (87) lobes. This demonstrates the differential distribution of high affinity calcium antagonist binding sites in fresh human brain cortex.

In another series of experiments, we observed that the saturation curves of [$^3$H]PN-200-110 in cortical samples obtained at autopsy (delays of 12-48 hrs after death) are biphasic suggesting the existence of two population of sites for this ligand. However, our data obtained from fresh human brain tissues indicate that the appearance of the low affinity site is probably an artifact related to autopsy delays. This reveals the difficulty in using autopsy tissue to study human brain calcium channel antagonist binding sites.

325.11 HUMAN GAMMA-GLOBULIN AND ALPHA-GLOBULIN DECREASE LIGAND BINDING AT NEUROTRANSMITTER RECEPTORS. A. C. Andorn*, F. Klemens*, P. Martello* and M. Pappolla*. ( SPON: M. Maguire) Dept. of Psychiatry, Case Western Reserve Univer. Schl. of Med. and Cleveland Metropolitan General Hospital, Cleveland, OH, 44109.

[$^3$H]Spiroperidol binds specifically at dopaminergic sites in human caudate and at multiple sites with predominant serotonergic selectivity in human prefrontal cortex (Andorn, A.C. in Usdin, E and Riederer, P. (edts) Neurotransmitter Biochemistry of the Human Brain, MacMillan, 183, 1981; Andorn, A.C. Huang, E. H. and Warren, A. Life Sci., 34, 2461, 1984). Human gamma-globulin and human alpha-globulin decrease the specific binding of this ligandin both caudate and prefrontal cortex in a dose-dependent fashion. The mean $IC_{50}$ for gamma-globulin in cortex was 3.4 ± a standard deviation of 0.8 mg/ml assay (N=12). In caudate the $IC_{50}$ was 2.8 ± 0.5 mg/ml assay (N=6). The mean $IC_{50}$ for human alpha-globulin in the cortex was 1.1 ± 0.1 mg/ml assay (N=6) and in caudate was 1.4 ± 0.8 mg/ml assay (N=6).

The effect of both gamma-globulin and alpha-globulin was due to a direct effect on the binding sites in that maximal decrements in [$^3$H]spiroperidol binding could be produced by preincubation of the particulate protein in globulin followed by removal of free globulin with extensive washing of the tissue. The effect of the globulins was therefore not due to $^3$H-ligand serum protein interactions. The effect of gamma- and alpha-globulin was selective in that it was not mimicked by human serum albumin, and further, preincubation of the tissue in human serum albumin did not prevent the effect of either gamma- or alpha-globulin. Immunoglobulin A was effective at decreasing specific [$^3$H]spiroperidol binding in cortex with an $IC_{50}$ of 0.9 ± 0.3 mg/ml assay (N=3).

Aberrant serum proteins have been demonstrated in the neuropil in a variety of illnesses including presenile and senile dementia (Wisniewski, H. M. and Kozlowski, P.B., Annal. N. Y. Acad. Sci., 396, 119, 1982). We have also demonstrated the presence of gamma-globulins, alpha-globulins and various immunoglobulins, in the neuron itself, in the axons and in the Lewy bodies associated with Parkinson's disease. These findings suggest that macromolecular leaks of the blood-brain barrier that permit the extravasation of serum proteins may have important consequences for neurotransmitter receptor function.

325.12 NALOXONE PREVENTS HYPERTHERMIA-INDUCED CONVULSIONS AND DEATH IN THE RAT PUP. M.M.Puig and M.L.Laorden.*
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The possibility that endogenous opioid peptides (EOP) are involved in producing febrile convulsions in children was studied in a rat pup model in which the effect of the opioid-antagonist naloxone (Nx) was tested in hyperthermia-induced seizures. Unrestrained 15-day-old male Sprague-Dawley rats were placed in a ventilated constant temperature chamber at 40°C and 55% humidity. Rectal temperatures were measured with a thermistor permanent probe connected to a recording thermometer. Three groups of 15 pups from different litters were randomly sampled and injected intraperitoneally with: 1) 0.2 ml saline (S), 2) 5 mg/kg or 3) 10 mg/kg of Nx. Temperatures were recorded after injection immediately after placing the pups in the chamber (time 0), and at 10 min intervals for 60 min; animals were observed for onset of generalized seizures and/or death. In group 1 (S), temperature rose from 35.7 ± 0.53°C (mean °C ± SE) at time 0, to 41.9 ± 0.10 °C at 60 min. Seizures were observed in 13% of the animals at 30 min (mean temp. 40.8 ± 0.19 °C) which rose to 93% at 40 min (41.5 ± 0.3 °C) and 100% at 50 min (42.0 ± 0.10 °C). At 50 min, 66% deaths were observed which increased to 86% at 60 min. Conversely, in group 3 (10 mg/kg Nx), no seizures nor deaths were observed at any time point after exposure to increased temperature, and temperatures were significantly lower than controls, at 30, 40 and 50 min. (35.5 ± 0.21 at time 0; 38.7 ± 0.20 at 30 min; 39.5 ± 0.15 at 40 min; 40.5 ± 0.15 at 50 min and 41.0 ± 0.10 at 60 min). Group 2 (5 mg/kg Nx), although also showing decreased temperature at 30 to 50 min when compared to group 1 (S), was partially protected from seizures (67% at 40 min versus 93% in controls) and death (46% at 50 min versus 66% in controls). No seizures nor deaths were observed when the same protocol and Nx dosages were repeated using randomly selected groups of 5 rats at a chamber temperature of 27°C.

Our results show that high dosages of naloxone completely prevent hyperthermia-induced seizures and death in the rat pup. These results indicate that: 1) EOP participate in the etiology of hyperthermia-induced seizures; 2) due to the high naloxone dosages required, EOP receptors other than mu may be involved and 3) based on (2), the EOP released is probably not a strong mu-agonist. These results suggest a possible clinical use of Nx in febrile convulsions in children and other hyperthermic states.

## GABA AND BENZODIAZEPINES: RECEPTOR CHARACTERIZATION AND LOCALIZATION II

326.1 MONOCLONAL ANTIBODIES TO A $GABA_A$/BENZODIAZEPINE RECEPTOR COMPLEX : HIGH RESOLUTION MAPPING IN RAT AND HUMAN BRAIN. J.G. Richards, P. Schoch* and H. Möhler*, Pharmaceutical Res. Dept., F. Hoffmann-La Roche & Co., Ltd., CH-4002 Basel, Switzerland.

The receptors mediating the therapeutic effects of benzodiazepines are localized exclusively in neuronal membranes of the CNS as part of an oligomeric complex with $GABA_A$ receptors and their associated chloride channels ; the receptor complex is presumed to be a tetramer composed of two α(50kd) and two β(55kd) subunits.

To date, attempts to map the distribution and density of benzodiazepine receptors in the CNS have been dominated by radiohistochemical techniques whose resolution, however, is limited by radiation scatter and possible diffusion of radioligands.

Species and subunit-specific monoclonal antibodies(mAb), raised against a purified $GABA_A$/benzodiazepine receptor from bovine cerebral cortex(Schoch, P. et al., Nature 314:168, 1985 ; Häring, P. et al., Proc. Natl. Acad. Sci. USA, in press), have now been used to map the receptor complex by immunohistochemical techniques(PAP method) with a far superior resolution. The distribution and density of receptor antigenic sites in rat brain, spinal cord and retina as well as in post-mortem human brain was similar to that of benzodiazepine and GABA receptors radiolabelled with $^3$H-Ro 15-1788 and $^3$H-muscimol respectively. The immune reaction(surrounding neuronal perikarya and dendrites) was extremely intense in cerebral cortex, olfactory bulb(particularly the external plexiform layer), ventral pallidum, islands of Calleja, globus pallidus, hippocampus, dentate gyrus, substantia nigra reticulata, cerebellum(granular > molecular layer), spinal cord(dorsal horn and around the central canal) and retina(in four distinct bands in the internal plexiform layer and around isolated amacrine cells) but was absent in brain white matter, pineal, pituitary, adrenals and superior cervical ganglia. The subunit-specific mAb showed an identical distribution in the CNS. These observations indicate that most benzodiazepine receptors are colocalized with the $GABA_A$ receptor complex. Accordingly, the appearance of antigenic sites in rat brain during ontogeny was found to coincide with that of the radiolabelled receptor complex.

Furthermore, in subcellular studies of the rat substantia nigra, for example, the immune reaction was localized exclusively in the pre- and postsynaptic membranes of axosomatic and axodendritic contacts.

The mAb are currently being used(together with GAD mAb) for the subcellular localization of receptors in defined GABAergic synapses and for the identification of possible pathological alterations of the receptor complex in human CNS diseases, such as epilepsy.

326.2 BENZODIAZEPINE RECEPTOR: IMMUNOCHEMICAL AND IMMUNOCYTOCHEMICAL CHARACTERIZATION. J.-Y. Wu, H. S. Lin, C.-T. Lin, Y. Xu, J.-W. Liu and S. C. Wei. Dept. of Physiology, The Penn State Univ., College of Med., Hershey, PA 17033.

The crude mitochondrial fraction, P2 membrane, from rat brain was used as the starting material for benzodiazepine receptor (BZR) purification. The purification procedures involved the covalent labeling of BZR by photoaffinity labeling using [$^3$H]-flunitrazepam (FNZP) as ligand, followed by solubilization and analysis on SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and finally by repetitive preparative SDS-PAGE. The purified BZR migrated as a single protein band on analytical SDS-PAGE corresponding to a molecular weight of 50,000-dalton. Furthermore, the position of the purified BZR on analytical SDS-PAGE matched with that of [$^3$H]FNZP-labeled BZR. The purified BZR preparations were used for the production of polyclonal as well as for the screening of monoclonal antibody against BZR. Antibodies against BZR could be detected in rabbits after three bi-weekly injections of 50 μg each of purified BZR. The specificity of the antibodies was established from immunodiffusion and Western blot transfer tests in which only one single precipitin band and one protein band with 50,000-dalton was obtained when either partially purified or purified BZR preparation was used. The specificity of monoclonal antibody was established by ELISA test using purified receptor preparation as antigen and by Western blot transfer test using either purified or partially purified BZR preparations as described above. Preliminary immunocytochemical studies using polyclonal antibodies and indirect peroxidase techniques have revealed that BZR is localized primarily on the postsynaptic membrane of the neurons receiving GABAergic innervation such as Purkinje cells in the cerebellum, pyramidal cells and some interneurons in the hippocampus. No staining in glial cells was observed. The localization of BZR from our immunocytochemical studies is consistent with the notion that BZ and GAGA systems are closely linked structurally and functionally (Supported in part by PHS grant NS 20922).

326.3 ALTERATION IN BENZODIAZEPINE RECEPTORS CAUSED BY GLYCOSIDASES. Paul Sweetnam and John F. Tallman, Dept. of Psychiatry, Yale University School of Medicine and Abraham Ribicoff Research Facilities of the Connecticut Mental Health Center, New Haven, CT 06508

The ability to photolabel benzodiazepine receptors in vitro from various regions of the rat brain with $^3$H-flunitrazepam has allowed for the structural examination of these receptors by Sodium dodecyl sulfate polyacrylamide gel electophoresis (SDS PAGE) under reducing conditions. Results for all regions studied revealed the labelled receptor to consist of a single major band of radioactivity with the apparent molecular weight of approximately 50K. Under our conditions of labeling we do not significantly label any higher molecular weight forms of the receptor. Exposure of the benzodiazepine receptors to the glycosidases, neuraminidase (N) or endoglycosidase H (E), results in the specific removal of sialic acids (N) and complete asparagine-linked carbohydrate moieties (E), respectively. This type of structural modification of the receptor resulted in a decrease in the the molecular weight, as determined by increased mobility, for all regions examined (Cortex+N+E, 8-10K; Hippocampus+N, 7K,+E, 12K; Cerebellum+N, 0K,+E 4K). These results point to a heterogeniety in the posttranslational glycosylation of the benzodiazepine receptor. This may be due to brain region specific differences in glycosylation.

A causal relationship has also been established between the removal of these carbohydrate moieties and a subsequent alteration in agonist/antagonist benzodiazepine binding. Cortical agonist binding following either glycosidase treatment resulted in no apparent shift in the $K_d$, but a significant decrease in the $B_{max}$. The $B_{max}$ change may be the result from a large decrease in affinity or denaturation of a subpopulation of benzodiazepine receptors. Antagonist binding also showed no apparent $K_d$ shift, but a significant increase in the $B_{max}$. The increase may have resulted from the activation of "hidden" benzodiazepine receptors or a shift of low affinity sites to sites of higher affinity. Cerebellum agonist or antagonist binding was not altered, in terms of either $K_d$ or $B_{max}$, by either enzyme treatment, correlating well with the small amount of carbohydrate removal seen following such treatments.

The ability of these enzymes to modify the apparent molecular weight of the benzodiazepine receptors and the strong correlation to altered ligand binding, in a regional specific manner, generally parallels the description given of type-1 and type-2 benzodiazepine receptors. These results indicate that differing glycosylation mechanisms may alter binding specificity of the receptor in such a manner as to be consistent with idea that regional distribution of type-1 and type-2 sites are due in part to carbohydrate composition of the receptor.

326.4 IMAGING BENZODIAZEPINE RECEPTORS IN MAN WITH C-11-SURICLONE AND POSITRON EMISSION TOMOGRAPHY. J.J. Frost*, R.F. Dannals*, H.T. Ravert*, A.A. Wilson*, J.M. Links*, R. Triffiletti, M.J. Kuhar, S.H. Snyder, and H.N. Wagner, Jr. (SPON: M. Lo). The Johns Hopkins Medical Institutions, Baltimore, MD.

Suriclone is a potent cyclopyrrolone, anti-anxiety drug which binds to the benzodiazepine receptor complex (BZR) with high affinity. Suriclone binds to a site on the BZR distinct from the site where benzodiazepines bind. The KD of suriclone at 37°C is 0.03 nM. C-11-suriclone (SUR) was synthesized by reacting C-CH3I with the appropriate amine precursor. SUR (1 ug/kg) was injected IV into a baboon alone or with 1 mg/kg of Ro 15-1788, a benzodiazepine antagonist, and serial PET scans of the brain were obtained. High radioactivity concentrations were observed in the cerebral cortex and cerebellum which contain high densities of BZR, intermediate concentrations in thalamus and low concentrations in the striatum. When Ro 15-1788 was given a uniform distribution of radioactivity was observed; the radioactivity was reduced to ca. 25% of control values in the brain which was contained within the PET slice.

SUR (0.2 ug/kg) was next administered to a human subject. From 30-60 minutes after injection high radioactivity concentrations were observed in the cerebral cortex and cerebellum, intermediate concentrations in the thalamus and a low concentration in the caudate. Radioactivity in the cerebral cortex and cerebellum decreased slowly with time, implying that binding of SUR to a high affinity site had occurred.

These results demonstrate utility of SUR for measuring binding to the benzodiazepine receptor complex non-invasively in man. Displacement studies in normal volunteers and studies in patients with selected neuropsychiatric disorders are in progress.

326.5 GABA B RECEPTOR-MEDIATED INHIBITION OF cAMP CONTENT IN PRIMARY CULTURES OF CEREBELLAR GRANULE CELLS. J. Xu* and W. J. Wojcik (SPON: J. Cohen). Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hospital, Washington, D.C. 20032.

We have previously reported a loss of GABA B receptors from cerebella of neurologically mutant mice deficient in granule cells. This indirectly suggested that a population of GABA B receptors could be associated with this cerebellar cell type. We now prepare primary cultures of cerebellar granule cells and use these cultures as a model system to determine the presence of GABA B receptors. Since the GABA B receptors have been shown to affect adenylate cyclase, we measured GABA B receptor-mediated effects on cAMP content from intact cells.

Granule cell cultures were prepared from cerebella of 8-9 day old Sprague-Dawley rat pups. The cells were cultured for eight days during which time the cells produced extensive neurites. On the eighth day, the cultures were tested by incubating them in an appropriate buffered salt solution. The cellular cAMP content was measured by a fluorometric-HPLC method. The cAMP phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine (IBMX), was included in every experiment to prevent the metabolism of cellular cAMP. The effect of baclofen and IBMX on cAMP content was not different from that seen with IBMX alone. In fact, the addition of IBMX slightly increased cAMP content, indicating a very low basal adenylate cyclase activity. Since we have previously observed only an inhibitory effect of baclofen on adenylate cyclase, the low basal adenylate cyclase activity did not allow us to observe any inhibition of cyclase by baclofen. Therefore, we added forskolin to the incubation to stimulate cAMP production. With 10 uM forskolin, cAMP content increased linearly during the first nine minutes of incubation. Baclofen (1 mM) was capable of attenuating the forskolin-stimulated cAMP accumulation by 50-60%. The EC 50 for baclofen was 5 uM and concentrations greater than 200 uM were maximally effective. It appeared that baclofen was interacting with the GABA B receptor since the rank order of agonist potencies, tested at 100 uM, was (-)baclofen (±) baclofen GABA, while the (+)baclofen, isoguavacine and muscimol were non-responsive. Furthermore, the inhibitory action of 100 uM GABA and (-)baclofen could not be antagonized by 100 uM bicuculline. Other receptor agonists as PGE1, isoproterenol and serotonin were found to have no effect on basal cAMP and/or forskolin-stimulated cAMP content. However, these cultures contained the cholinergic muscarinic receptor, since carbachol and oxotremorine decreased cAMP content.

In summary, the presence of GABA B receptors have been demonstrated on cerebellar granule cells in culture. Our results prove that agonists at the GABA B receptor can attenuate cAMP formation.

326.6 ISLET ACTIVATING PROTEIN (IAP) PREVENTS GABA B RECEPTOR-MEDIATED INHIBITION OF ADENYLATE CYCLASE. W. J. Wojcik and J. Xu*. Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hospital, Washington, D.C. 20032.

We have shown GABA and baclofen, a GABA analog, to inhibit basal and forskolin-stimulated adenylate cyclase activity in a crude synaptosomal membrane preparation from various brain regions. This effect is believed to be mediated through a GABA B receptor coupled to an inhibitory guanine nucleotide (Ni) regulatory unit of adenylate cyclase. Agonists of the GABA B receptors inhibit via activation of the Ni unit adenylate cyclase activity. Primary cultures of cerebellar granule cells were the source of GABA B receptors coupled to adenylate cyclase for these studies. Using crude membrane preparation from these cultured cells, we showed that the baclofen-mediated response requires GTP. The Km for GTP was about 0.5 μM in the presence of a maximal concentration of baclofen (300 μM). The observed Km of GTP is similar to that of other receptors coupled to Ni linked to adenylate cyclase. It is of interest that the GTP curve was monophasic, having only a stimulatory phase on cyclase activity. This presumably reflects the activation of the stimulatory nucleotide unit. There did not appear any downward deflection with higher concentrations of GTP.

We also show a loss in the baclofen inhibitory response to cyclase after treating the cells with IAP inactivates the Ni unit by an ADP-ribosylation of the Ni. After a 14 hour exposure to IAP (330 ng/ml, List Biologics), we observed a 52% loss in the baclofen-mediated inhibition of basal adenylate cyclase activity and a 60% loss in the baclofen inhibition of forskolin-stimulated cyclase. Similar treatment with IAP also antagonized the baclofen induced decrease of forskolin-stimulated cAMP accumulation by 67% in intact granule cells in primary culture. In conclusion, baclofen inhibits adenylate cyclase activity by stimulating GABA B receptors coupled to the Ni unit of adenylate cyclase.

326.7 BIOCHEMICAL AND FUNCTIONAL CHARACTERIZATION OF GABA-MODULIN IN A NEURONAL CELL POPULATION. F. Vaccarino, H. Alho, A. Guidotti and E. Costa. Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hospital, Washington, D.C. 20032.

GABA-modulin is a brain basic protein that appears to down regulate GABA receptors allosterically, in vitro. In subcellular studies of rat brain homogenates, GABA-modulin migrates with the synaptosomal fraction, moreover it can be differentiated biochemically from the basic proteins present in myelin (J. Neurochem. 44: 278, 1985). To study the location and function of GABA-modulin, an antiserum was raised in rabbits that contain an antibody highly specific for GABA-modulin (50% tracer bound with ? pmol of protein) but shows some cross reactivity with basic myelin proteins. In order to eliminate this cross-reactivity, the antiserum was pre-absorbed with an excess of myelin basic proteins. After pre-absorption, no immunoreactivity was detected with purified myelin basic proteins (up to 70 pmoles) or with a crude extract of rat brain myelin after HPLC fractionation, while a crude extract of synaptosomes analyzed through HPLC showed a major immunoreactive peak which coeluted with purified GABA-modulin. Immunoreactivity was also detected in an enriched population of granule cells prepared from cerebellum of neonatal rats. Granule cells in primary culture are not myelinated and contain approximately 5% glial cells (Developm. Brain Res. 10: 227, 1983). Immunohistochemical studies in the granule cell culture with the antiserum against GABA-modulin preabsorbed with basic myelin proteins failed to reveal any staining in the glial cells while granule cells were very brightly stained with a pattern characteristic of neuronal membrane proteins. HPLC fractionation of a crude granule cell extract revealed a single immunoreactive peak coeluting with purified GABA-modulin; SDS-PAGE separation of the same cell extract followed by immunoblotting showed a major immunoreactive band with molecular weight identical to GABA-modulin. The material purified from granule cell cultures is identical in molecular weight (17,000), amino acid composition, biological activity and immunoreactivity characteristics with GABA-modulin purified from synaptosomes. Granule cells contain 1.2 µg/mg protein of GABA-modulin-like protein.

Intact granule cells in culture express all the components of the GABA/benzodiazepine/$Cl^-$ receptor complex; moreover, GABA receptors are up-regulated by benzodiazepines and down regulated by GABA-modulin. Thus, these cultures represent an excellent model for studying in detail the mechanism of action of GABA-modulin on the regulation of GABA receptors.

326.8 NEURONAL LOCATION OF A BRAIN NEUROPEPTIDE (DBI) PUTATIVE PRECURSOR OF THE ENDOGENOUS LIGAND FOR BRAIN BETA-CARBOLINE RECOGNITION SITES. H. Alho, P. Ferrero*, A. Guidotti and E. Costa. Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hosp. Washington, D.C. 20032

An endogenous neuropeptide (DBI) that displaces β-carbolines from their specific brain recognition sites has been isolated and purified from rat and human brains (Guidotti et al., PNAS 80: 3531, 1983). DBI injected intraventricularly into rats facilitates conflict in Vogel's behavioral tests.

Immunohistochemical studies were conducted with a DBI antiserum raised in rabbits. Its affinity and specificity were documented with conventional techniques. In rat brain, DBI immunoreactivity is highly localized in nerve terminals of several telediencephalic and amygdaloid nuclei, in pyramidal and basket cells of hippocampus and in cells and fibers of the cerebellar molecular layer. Even without colchicine treatment, some cells in the hippocampus and cerebellum could be visualized. In rats receiving kainic acid (3.7 nmol icv) 6 days before sacrifice DBI immunoreactive staining of cells and fibers disappeared from specific hippocampal areas revealing the neuronal localization of DBI. In specific brain structures the immunohistochemical location of DBI is very similar to that of GABA especially in the molecular layer of cerebellum and in hypothalamic nuclei, but DBI may be present also in neurons that are not GABAergic.

DBI contains two replicas of an octadecaneuropeptide (ODN) (18 amino acids) suggesting that DBI may function as a precursor of ODN. The latter may act as an endogenous ligand for benzodiazepine recognition sites. The ODN has been sequenced and synthesized and an antiserum against the ODN was produced in rabbits. This serum exhibits a slight cross reaction with DBI but fails to bind with several other neuropeptides. The ODN antisera preabsorbed with the synthetic peptide showed no specific immunostaining. The ODN immunoreactivity was localized mainly in brain areas with DBI immunostaining. For instance, ODN immunoreactive neurons were found in pyramidal cells of hippocampus and cerebral cortex and in cells and fibers of the molecular layer of cerebellum.

In conclusion DBI and ODN are located in selected neuronal populations of rat brain. DBI may be a precursor of a new family of brain neuropeptides that bind to benzodiazepine/β-carboline recognition sites and may have a physiological role in regulating the behavioral patterns elicted by conflict situations (anxiety or aggression).

326.9 STRUCTURE-ACTIVITY RELATIONSHIP OF NEUROPEPTIDES DERIVED FROM DBI, THE PRECURSOR FOR BRAIN ENDOGENOUS LIGANDS OF β-CARBOLINE RECOGNITION SITES. R. Santi*, A. Guidotti and E. Costa. Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hospital, Washington, D.C. 20032.

An octadecaneuropeptide (ODN) obtained by tryptic digestion of DBI was purified and shown to have the following structure: Gln-Ala-Thr-Val-Gly-Asp-Val-Asn-Thr-Asp-Arg-Pro-Gly-Leu-Leu-Asp-Lys. This structure was confirmed by synthesis and HPLC characterization of the natural and synthetic compound. In vitro, synthetic and natural ODN displaced $^3$H-beta-carbolines binding from specific recognition sites of brain synaptic membranes (Neuropharmacology 23: 1359, 1984). However the use of crude synaptic membranes from brain represents a limitation for neuropeptide binding studies because of their high proteolytic activity. Hence we used neuronal cell cultures of rat neonatal cerebellar interneurons enriched in granule cells (Dev. Brain Res. 10: 277, 1982). These cells in addition to GABA and $Cl^-$ channel receptor sites contain high affinity ($K_D$ 3.5 nM) and saturable (Bmax 450 fmol/mg protein) $^3$H-methyl-beta-carboline-3-carboxylate (beta-CCM) binding sites. We have found that ODN added to this cerebellar interneurons, maintained in Locke's solution at pH 7.4, displaces $^3$H-beta-CCM from its binding sites with a Hill slope close to 1, and a Ki of 4 µM and 1.5 µM at 18° and 0-4°, respectively. ODN appears to act competitively. The α-amide derivative of ODN fails to displace $^3$H-beta-CCM. In contrast, smaller synthetic peptide fragments of ODN such as: Arg-Pro-Gly-Leu-Leu-Asp-Leu-Lys (octapeptide); Pro-Gly-Leu-Leu-Asp-Leu-Lys (heptapeptide) and Gly-Leu-Leu-Asp-Leu-Lys (hexapeptide) displace $^3$H-beta-CCM binding with a Ki ranging from 5 to 10 µM. Two other DBI fragments structurally unrelated to ODN, including a heptamer (Phe-Ile-Tyr-Ser-His-Phe-Lys) and a tetramer (Thr-Tyr-Val-Glu) failed to displace $^3$H-beta-CCM from its binding site to cerebellar granule cells in concentrations up to 50 µM. The ODN contains the sequence responsible for the $^3$H-beta-CCM displacing activity of DBI. Moreover the studies with smaller fragments of ODN and ODN α-amidated derivative reveal that within the ODN structure the activity is located in the last eight to six carboxylterminal amino acids. We are now testing the displacing activity of several other ODN derivatives and their stereoisomers in order to determine the minimal structural requirement for this biological activity.

326.10 DMCM (methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate) SEIZURES DISCRIMINATE SENSITIVELY BETWEEN VARIOUS TYPES OF BENZODIAZEPINE RECEPTOR LIGANDS. E.N. Petersen, T. Honoré and L.H. Jensen. A/S Ferrosan, Research Division, Sydmarken 5, DK-2860 Soeborg, Denmark (SPON: European Neuroscience Association)

DMCM is a convulsant characterized as an inverse agonist at BZ receptors (Petersen, Eur.J.Pharm., 94:117, 1983). DMCM is convulsant in mice at doses displacing about 50% of $^3$H-flunitrazepam ($^3$H-FNM) from its receptors in vivo (Braestrup et al., Science, 216:1241, 1982). Benzodiazepine (BZ) receptor ligands from several chemical classes have been studied for antagonism of DMCM-induced and pentylenetetrazol (PTZ)-induced seizures and compared to their in vivo displacement of $^3$H-FNM. No correlation was found between the DMCM and PTZ antagonisms further substantiating the different mechanisms of action of these convulsants. Furthermore, we found a wide scattering among the 90 compounds tested with respect to the DMCM antagonism and the $^3$H-FNM displacement in vivo. Generally the BZ were found unexpectedly weak against DMCM seizures as 50-90% BZ receptor occupancy was necessary to obtain protection. Premazepam (MDL 181) was found inactive even at doses occupying >97% BZ receptors (300 mg/kg i.p.). The BZ receptor antagonists Ro 15-1788 and ZK 93426 as well as the partial inverse agonist CGS 8216 were more effective than most BZ as only 50-60% BZ receptor occupancy was required for protection. Partial inverse agonists of the β-carboline type were moderately to weakly active against DMCM-induced seizures. Some agonistic β-carbolines (PTZ antagonists) exerted surprisingly potent DMCM antagonism (<10% BZ receptor occupancy). Braestrup et al., J.Neurochem., 41:454, 1983 have provided evidence for a deviating BZ receptor binding profile of $^3$H-DMCM compared to $^3$H-FNM. The present results suggest that compounds may antagonize DMCM induced seizures by interference with the DMCM binding site (a BZ site) either by direct competition or allosterically (BZ receptor antagonists and benzodiazepines?) or a combination of binding and efficacy of the ligand at the DMCM binding site (β-carbolines?). Downstream inhibition of the expression of the DMCM induced seizures by a DMCM binding site unrelated mechanism may also occur as found with the antiepileptic valproate. Moreover the results show that only some β-carbolines possess unexpectedly high potency against DMCM seizures suggesting that the DMCM binding site is not just a β-carboline site but might be a BZ receptor subtype with high structural specificity. The BZ apparently do not fit to this subtype very well at body temperature and the atypical BZ premazepam apparently has no affinity for this DMCM sensitive subtype even in doses displacing >97% of $^3$H-FNM in vivo.

326.11 ZOLPIDEM (SL 80.0750-23N) : A NOVEL NON-BENZODIAZEPINE HYPNOTIC. S.Z. Langer, H. Depoortere*, S. Arbilla* and P. George*. Department of Biology, Laboratoires d'Etudes et de Recherches Synthélabo (L.E.R.S.), 58, rue de la Glacière, 75013 Paris, France

Zolpidem (SL 80.0750-23N : N,N,6-trimethyl-2-(4-methylphenyl)imidazo[1,2-a]pyridine-3-acetamide hemitartrate), is an imidazopyridine derivative, which after oral or i.p. administration to rats and cats produces a rapid onset short acting sedative effect. Zolpidem displaces with different affinities $^3$H-diazepam binding to membranes of rat cerebral cortex ($IC_{50}$ = 60 nM), cerebellum ($IC_{50}$ = 17 nM) and hippocampus ($IC_{50}$ 109 nM). Zolpidem has low affinity for the peripheral benzodiazepine (BZD) receptor in the rat kidney labelled with $^3$H-Ro-5-4864 ($IC_{50}$ = 1900 nM). The affinity of zolpidem for the central BZD receptor in the rat cortex labelled with $^3$H-Ro-15-1788 ($IC_{50}$ = 141 nM) is significantly enhanced in the presence of GABA 100 µM (ratio $IC_{50}$ - GABA / $IC_{50}$ + GABA = 3.35 + 0.27, n = 4), indicating an agonist type of interaction with high intrinsic activity at the BZD receptor. In cortical recordings of curarised rats, zolpidem (0.3 mg/kg p.o.) disrupts the pattern of wakefulness and induces sleep periods of short duration. In the range of 0.1 - 1 mg/kg i.p., zolpidem induces sleep periods of 10 - 60 min in a dose-dependent manner. At 0.1 and 1 mg/kg (i.p.), zolpidem induces large slow wave sleep, with some sleep spindles at 12 - 14 Hz. In marked contrast with the profile observed with BZDs, the power spectral analysis of the electrocorticogram recordings with increasing doses of zolpidem (0.1 - 1 mg/kg i.p.) show fast onset transient increases in the energy frequency bands 1 to 16 Hz and a dominant peak in the delta frequency band (1 - 4 Hz). The sleep pattern of zolpidem is however mediated through BZD receptors as it is antagonized by Ro 15-1788. No rebound effects occur on withdrawal. In freely moving cats chronically implanted with electrodes, zolpidem does not affect total duration of the different sleep phases (Arbilla S. et al., Br. J. Pharmacol., 1985, in press). In man, zolpidem is an hypnotic which increases the duration of slow wave sleep and does not affect rapid eye movement sleep (Nicholson A.N. and Pascoe D.A., Br. J. Pharmacol., 1985, in press). In summary, our results indicate that zolpidem, which is chemically different from BZDs, activates central but not peripheral BZD receptors. Zolpidem possesses BZD receptor agonist properties, and has a higher affinity for cerebellar than hippocampal BZD recognition sites. In addition, zolpidem is a short acting hypnotic, which induces a physiological pattern of slow wave sleep. Therefore, imidazopyridines like zolpidem represent a novel class of agonists at BZD receptors, which possess clear pharmacological differences and potential therapeutic advantages over classical BZDs.

326.12 NEUROPHARMACOLOGICAL AND BEHAVIOURAL EFFECTS OF ZOLPIDEM, A NOVEL SHORT-ACTING HYPNOTIC. B. Zivkovic*, K.G. Lloyd, D.J. Sanger*, G. Perrault*, E. Morel*, S.Z. Langer and G. Bartholini*. Laboratoires d'Etudes et de Recherches Synthélabo (L.E.R.S), 58 rue de la Glacière, 75013 - Paris, France.

Recent studies have demonstrated that zolpidem (SL 80.0750-23N; N,N,6-trimethyl-2-(4-methylphenyl) imidazo [1,2-a] pyridine-3-acetamide) potently displaces benzodiazepine receptor ligands from their central recognition sites and induces a rapid onset, short-lasting hypnotic effect in experimental animals and man (Langer et al., this meeting; Nicholson and Pascoe, Br. J. Pharmacol. 1985, in press). The present studies were performed in order to establish the neuropsychopharmacological profile of the compound and to compare its spectrum of activity with that of benzodiazepines.

Qualitatively, zolpidem shares some properties with benzodiazepines (Table); these effects were antagonised by the benzodiazepine antagonist Ro15-1788, indicating involvement of benzodiazepine sites in the action of zolpidem.

| | Pentetrazole$^+$ Tonic Convul. Mouse | Loaded Grid$^+$ Test Mouse | Conflict* Drinking Rat | Rota-Rod$^+$ Rat |
|---|---|---|---|---|
| Zolpidem | 7.7 | 17 | 1 | 6 |
| Midazolam | 0.5 | 1 | 1 | 3 |
| Flunitrazepam | 0.05 | 0.1 | 0.3 | 0.6 |
| Diazepam | 0.4 | 1 | 3 | 5 |

$^+ED_{50}$ (mg/kg, i.p.); *Minimal effective dose (mg/kg, i.p.).

However, zolpidem differs from benzodiazepines in various aspects. Thus, in spinalised rats zolpidem reduced the hind limb flexor reflex only when injected at doses which induce narcosis (10 mg/kg, i.v.) while diazepam is active at 2 mg/kg, i.v. In decerebrate cats zolpidem enhanced dorsal root potentials with a potency similar to that of diazepam ($ED_{50}$=0.3 mg/kg, i.v.) and duration of action similar to that of midazolam (half life of the effect 25 min). However, in contrast to diazepam, this effect was not accompanied by a corresponding presynaptic inhibition of the monosynaptic reflex recorded from the L-7 ventral root.

In rats trained to discriminate chlordiazepoxide (5 mg/kg, i.p.) from saline, zolpidem failed to generalize with the chlordiazepoxide-associated-lever up to a dose (3 mg/kg, i.p.) which induced a strong decrease in the overall rate of responding, indicating that the compound and benzodiazepines do not share the same discriminative stimulus properties.

Together, these results demonstrate that zolpidem possesses anticonvulsant and anxiolytic activities, but in contrast to benzodiazepines is virtually devoid of myorelaxant properties.

## BRAIN METABOLISM III

327.1 DURATION OF 2-DEOXYGLUCOSE-6-PHOSPHATE (2dGlc-6-P) IN BRAIN by R.K. Deuel, T.W. Anderson* and R. Stephani,* Departments of Pediatrics and Neurology, Washington University School of Medicine, St. Louis, Missouri, 63110

$^{31}$P NMR spectroscopic monitoring of the time course of 2dGlc-6-P in brains of conscious rats revealed peak concentrations at 40-60 minutes after an iv bolus of 500 mg/kg, with decline to half the peak concentration by 120 min. (Deuel, et al, Science, in press). This time course is much shorter than that reported with $^{14}$C-labeled tracer doses of 2dGlc. To directly test if the 500 mg/kg dose induces a shorter half life of 2dGlc-6-P in brain than a tracer dose, NMR methodology could not be used as it would not detect very low concentrations of 2dGlc-6-P. An autoradiographic experiment was therefore performed. 250 mg conditioned rats given an 0.25 cc iv bolus containing 50 µCi/kg $^{14}$C-labeled 2dGlc, plus 500 mg/kg of unlabeled 2dGlc were paired with rats given an 0.25 cc bolus containing only the labeled tracer. Timed arterial samples were taken for scintillation counting, glucose assays, and hematocrits. Four pairs of rats were sacrificed at 50 minutes, four at 120 minutes and four at 240 minutes after bolus injection. Animals were perfused through the heart, and the brain rapidly removed, blocked and frozen. To determine if regional differences in the time course of 2dGlc-6-P existed, 30 µ frontal and parietal coronal sections were used to prepare autoradiograms that were analyzed by densitometry for tissue concentration of $^{14}$C. The remainder of each brain was solubilized and aliquots subjected to scintillation counting. In contrast to the increasing divergence of $^{14}$C tissue concentration over time that was predicted between pair members if the 500 mg/kg dose accelerated tissue 2dGlc-6-P hydrolysis, isotope concentration in whole brain as well as in selected grey matter structures showed a constant ratio between the pairs over time, suggesting that the large dose has no effect upon the duration of cerebral $^{14}$C-2dGlc-6-P. Supported by NIH grants GM-30331 and the McDonnell Center for Study of Higher Brain Function of Washington University.

327.2 NO EVIDENCE FOR IN VIVO DEPHOSPHORYLATION OF GLUCOSE-6-PHOSPHATE IN RAT BRAIN. G. Dienel, T. Nelson*, T. Jay* and L. Sokoloff. Laboratory of Cerebral Metabolism, NIMH, Bethesda, MD 20205

Quantitative trapping of deoxyglucose(DG)-6-phosphate during the 45 min experimental interval is assumed in the determination of local rates of cerebral glucose utilization by the [$^{14}$C]DG method (Sokoloff et al., J. Neurochem., 28:897, 1977). Recently, Huang and Veech (J. Biol. Chem. 257:11358, 1982) reported evidence for a very high rate of dephosphorylation of brain glucose-6-phosphate (G-6-P) on the basis of the differential rates of loss of $^3$H and $^{14}$C from brain glucose isolated at intervals after an intracarotid injection of a mixture of [2-$^3$H]- and [U-$^{14}$C]glucose. Detritiation of G-6-P at the hexosephosphate isomerase step followed by dephosphorylation of G-6-P will cause a progressive decrease in the $^3$H/$^{14}$C ratio of brain glucose with time after the injection. Huang and Veech isolated glucose from freeze-blown brain samples by passage of the neutralized brain extract sequentially through a Dowex AG1-X8 formate column and a Dowex AG1-X8 borate column; the borate eluate was defined as the glucose fraction without any proof of purity. Our laboratory, using more specific methods to purify brain glucose, could not confirm the differential loss of $^3$H compared to $^{14}$C from brain glucose (Nelson et al., Trans. Amer. Soc. Neurochem. 16:147, 1985). The purpose of this study was to determine the $^3$H/$^{14}$C ratios in brain glucose isolated according to the procedures of Huang and Veech. Rats were injected with a mixture of [2-$^3$H]- and [U-$^{14}$C]- glucose via the carotid artery, and were killed by freeze-blowing at about 7 or 9 min. after the injection. Deproteinized brain extracts were neutralized and passed over tandem Dowex AG1-X8 formate and borate columns. The $^3$H/$^{14}$C ratios of the borate-column eluate of the brain samples were significantly ($p < 0.01$) lower than those of the blood samples taken at kill time, and both were less (22% and 11%, respectively) than that of the injectant which was carried through the same extraction and purification steps. Thin layer chromatographic analysis of the borate column eluate demonstrated the presence of other radiolabeled compounds in addition to glucose. The borate-column eluates were then further purified by sequential (1) passage over Dowex-50 to remove basic metabolites, (2) derivitization of its glucose component to gluconic acid with glucose oxidase, and (3) separation of gluconic acid from neutral metabolites contained in the borate column eluate by passage over Dowex 1-formate. The $^3$H/$^{14}$C ratios of the gluconic acid derived from brain samples were slightly below that of the injectant, but they were no less than that of the blood samples. The slight decrease in the brain ratio can be ascribed to the glucose delivered to the brain by the blood. Thus, there is no evidence for detritiation in the brain glucose pool, and we confirm the results of Nelson et al. (1985).

327.3 DEMONSTRATION OF UNILATERAL ACTIVATION OF THE RAT SOMATOSENSORY SYSTEM BY MEANS OF A SEQUENTIAL DOUBLE-LABEL AUTORADIOGRAPHIC METHOD. J. L. Olds*, K. A. Frey, C. A. Bay* and B. W. Agranoff (SPON: J. Schacht). Neuroscience Lab/Cyclotron PET Facility, University of Michigan, Ann Arbor, MI 48109.

Most of the variability inherent in the autoradiographic imaging of LCMRglc by means of the 2-DG method can be attributed to inter-subject differences in global cerebral metabolism. Thus, for studies involving small changes in glucose utilization, one is obligated to use very large numbers of animals, which in many cases is a prohibitive factor. We recently addressed this problem by means of a quantitative sequential double-label autoradiographic method employing $^{18}F$- and $^{14}C$-labeled fluorodeoxyglucoses (Olds et al., Brain Res., in press). We report here the successful application of the sequential double-label method employing $^{3}H$- and $^{14}C$-labeled deoxyglucoses to demonstrate an activation of the somatosensory pathway in response to passive manipulation of the right forepaw. The described method takes advantage of the differential β-energies of the two isotopes and uses two autoradiographic exposures: one to LKB-Ultrofilm, and the other to Kodak SB-5 X-ray film. Self-absorption of tritium was minimized by a lipid extraction with hexane (Olds et al., Trans. Amer. Soc. Neurochem. 16:148, 1985). A pixel-by-pixel subtraction of the digitized images was employed to facilitate isotopic separation. Each 20 μm brain section thus produced two data sets: one representing LCMRglc under the resting state, and the other representing LCMRglc under the stimulated state. Unilateral mechanical manipulation enabled us to emphasize the differences in brain metabolism separated by the 2-state paradigm. We found that the experimentally perturbed condition produced activation of the somatosensory cortex contralateral to the stimulated limb, while the control condition led to a clearly distinguishable image from the same brain section. An analogous experiment revealed the anticipated pattern that follows auditory stimulation. This method makes possible a form of analysis not previously available in DG studies in which each animal serves as its own control. The method has particular applicability to the analysis of symmetrical changes in LCMRglc, in which the intrinsic variability of subject-to-subject regional brain metabolism might otherwise greatly reduce sensitivity. (Supported by NIH Grant NS 15655.)

327.4 CELLULAR AND SUBCELLULAR LOCALIZATION OF DEOXYGLUCOSE UPTAKE IN ANTIDROMICALLY STIMULATED SUPERIOR CERVICAL GANGLION. P. Yarowsky, A. Boyne and R. Johnson*. Dept. of Pharmcol. Exp. Therap., Univ. of Maryland Sch. of Med., Baltimore, MD 21201.

With the $^{14}C$ deoxyglucose method, we have previously demonstrated a correlation between impulse activity and enhanced postsynaptic metabolic activity in the rat superior cervical ganglion (RSCG) in vivo during antidromic stimulation of the external carotid (EC) nerve (Yarowsky et al., Brain Res. 334:330, 1985). The spatial resolution obtainable with $^{14}C$ does not permit an analysis of which postsynaptic elements were metabolically activated. We therefore have employed methods for both $^{3}H$ autoradiography and ultrastructural preservation of water-soluble compounds with quick freezing, freeze substitution and the 'dry' emulsion method of Nagata et al. (Histochemie 18:1077, 1969).

The collagenase-pretreated and desheathed RSCG was pulse-labeled with [$^{3}H$]deoxyglucose (2-DG) during antidromic stimulation of the EC nerve at 30° C, rinsed in Locke solution and then quick frozen, THF substituted, osmicated and embedded in Epon/Araldite. Light-level autoradiograms (LM ARG) were prepared by mounting 5 μm sections with a drop of glycerol on slides and applying a loop of K5D emulsion. EM autoradiographs (EM ARG) were made by cutting 90 nm sections onto glycerol. The sections were carbon-coated and looped with L4 emulsion.

LM ARG of antidromically stimulated tissue confirmed the localization of enhanced glucose utilization in the caudal pole of the RSCG, previously seen with $^{14}C$ autoradiography. The pattern of enhanced uptake in the caudal pole was compared in three anatomical compartments, somata of neurons, somata of glia and neuropil, by superposition of the LM ARG with stained sections. The grain densities of the compartments were: neuropil, 8/100 $\mu m^2$; neuronal somata, 26/100 $\mu m^2$ glia somata 17/100 $\mu m^2$. Neither the rostral pole of the antidromically stimulated RSCG, an internal control, nor the caudal pole of unstimulated preparations demonstrated similar uptake.

In order to eliminate possible effects of afferent stimulation via the EC nerve, RSCG were chronically decentralized (4 d) prior to pulse labeling or pulse labeled either in the presence of Locke solution containing low $Ca^{+2}$ (0.2 mM) and high $Mg^{+2}$ (8 mM) or in Locke solution containing the ganglionic blocker hexamethonium (3 mM). No differences in the pattern or the grain density distribution were found.

Preliminary analysis of EM ARG from antidromic stimulated tissue showed several putative sources of radioactivity. These were, in order of the highest grain density: (1) neuronal cytoplasm (2) neuronal mitochondria (3) neuronal nuclei (4) glial cytoplasm and (5) dendrites. No labeling of afferent synapses, pre- or postsynaptically, was observed. (Supported by NSF grant BNS 81-19481.)

327.5 LOCAL CEREBRAL GLUCOSE UTILIZATION DURING GENERALIZED SEIZURES IN NEWBORN MONKEYS. D.G. Fujikawa, B.E. Dwyer*, R.R. Lake* and C.G. Wasterlain*. Epilepsy Res. Lab. and Nucl. Med. Serv., V.A. Med. Ctr., Sepulveda, CA 91343 and Dept. of Neurology and Brain Res. Inst., UCLA Sch. of Med., Los Angeles, CA 90024.

Recent evidence indicates that cerebral energy demands may exceed substrate supply during status epilepticus (SE) in newborn monkeys. Since this suggests increased brain glucose utilization we measured local cerebral metabolic rates for glucose (l-CMRgl) in newborn monkeys during bicuculline-induced seizures, using the $^{14}C$-2-deoxyglucose (2DG) autoradiographic technique. Cerebral cortical glucose concentrations were obtained in a separate group of control and 30 minute seizure animals with the funnel-freezing method (Fujikawa, D.G. et al., Neurology, 35 (Suppl.1):196, 1985). This allowed us to estimate the lumped constant (LC) in cerebral cortex with a nomogram (Pardridge, W.M. et al., J. Cereb. Blood Flow Metabol., 2:197-202, 1982), which permitted a more accurate estimation of cortical glucose utilization during seizures.

Six 2 week old marmoset monkeys had femoral vessel cannulations under methoxyflurane anesthesia, and after 4 hr of recovery they received ketamine, 50 mg/kg I.P., in accordance with the funnel-freezing protocol. Seizure animals (n=3) were given bicuculline, 10 mg/kg I.M., and when seizures began, 2DG (0.3 μCi/g) was given I.V. Control animals (n=3) were given 2DG 15-30 min after ketamine. The animals were decapitated after a 45 min interval of arterial blood sampling. Brains were processed for autoradiography and blood samples for liquid scintillation counting and plasma glucose determinations. Using the Pardridge nomogram, we found that the cortical LC was 0.42 in control and 1.0 in seizure animals. We used the 0.42 value to calculate l-CMRgl in all animals and the 1.0 value to correct the l-CMRgl in cortex during SE.

Most brain regions showed a 150-600% uncorrected increase in l-CMRgl during SE. Local CMRgl was unchanged in lateral geniculate nuclei, cerebellum and optic tract; it was decreased in inferior colliculi. In frontal cortex control l-CMRgl was 21.3 ± 2.6 μmol/100g/min (mean ± S.E.M.); it appeared to increase 4-fold during SE. When the LC value of 1.0 was used, the corrected l-CMRgl in frontal cortex was 35.6 ± 1.2 μmol/100g/min, a 170% instead of a 400% increase, but still significantly elevated ($p<0.01$).

Our results suggest that during neonatal SE glucose utilization in cerebral cortex increases almost 2-fold and that LC correction is essential to prevent overestimation of l-CMRgl. Together with our previous data showing preferential blood flow to brainstem and marked depletions of cortical glucose and high energy phosphates during neonatal seizures, our findings suggest that cortical energy demands and glucose utilization exceed glucose supply. This could lead to cortical energy failure and cell damage and could be the basis for the increased vulnerability of the immature brain to damage from SE. (Supported in part by NIH Grant NS-13515.)

327.6 FOCAL CEREBRAL ISCHEMIA: EFFECT OF $CO_2$ ON CEREBRAL BLOOD FLOW HETEREOGENEITY. B. Bose*, S.C. Jones*, H.T. Friel*, C. Chernicky, K.L. Barnes, J.R. Little*. Cleveland Clinic Foundation, Cleveland, OH 44106.

Previous investigators have observed temporal cycling in the cerebral cortex of cerebral blood flow (CBF), vascular volume, the level of metabolic substrates and the spatial microheterogeneity of CBF. The aim of this study is to determine the effect of normocapnia, hypocapnia and hypercapnia on the spatial heterogeneity of CBF in the rat middle cerebral artery occlusion model.

Sprague-Dawley rats (350-400 gms) were lightly anesthetized with pentobarbital, tracheotomized, and their right common carotid artery (RCCA) isolated. The femoral arteries and veins were cannulated bilaterally. The right middle cerebral artery (RMCA) was exposed and the RCCA and RMCA were occluded for a duration of two hours. At the end of this period $PaCO_2$ was adjusted to 25 mmHg, 35 mmHg, or 60 mmHg. After heparinization (100 IU), the rats were injected with boluses of 4% thioflavine S (0.7 mg/kg) 45 sec prior to sacrifice and $^{14}C$-iodoantipyrine (100 μCi/kg) 10 sec prior to sacrifice. An arterial blood sample was obtained for 30 sec prior to sacrifice with a calibrated withdrawal pump. The head was frozen in liquid Freon-22 after sacrifice by guillotine. Autoradiograms were made from 20 μm frozen brain sections. Alternate 20 μm sections were examined under fluorescent light to visualize the cortical microvasculature.

In normocapnic animals, regularly spaced columns of CBF perpendicular to the cortical surface were observed in the contralateral, non-infarcted (control) hemisphere. Several widely spaced columns were observed in the center of the severely ischemic area. In hypocapnic animals flow columns were visualized on the contralateral (control) side but were absent in ischemic areas of the infarcted hemisphere. In hypercapnic rats there was a homogeneous distribution of flow (no columns) on the control side; flow columns were present in the ischemic penumbra and umbra. This data demonstrating the presence of flow columns in severely ischemic areas in normocapnic and hypercapnic animals is contrary to previous findings and may be technique dependent. Furthermore, these columns seem to retain their $CO_2$ sensitivity to hypocapnia for a least two hours following the onset of a severe focal ischemic insult.

327.7 **HEMODYNAMIC AND METABOLIC ALTERATIONS FOLLOWING CORTICAL INFARCTION IN THE RAT.** W.D. Dietrich, M.D. Ginsberg, R. Busto*, and B.D. Watson*. Departments of Neurology and Anatomy and Cell Biology, Univ. of Miami School of Medicine, Miami, FL 33101.

Both clinical and experimental studies have demonstrated that the effects of focal brain injury are not restricted to the primary focus of damage, but may be quite diffuse and appear in brain regions remote from the primary injury site. Efforts to study stroke mechanisms responsible for widespread hemodynamic and metabolic abnormalities have been hampered by the lack of appropriate animal models. Recently we have developed in the rat a reproducible model of cortical infarction initiated by intravascular thrombosis. In the present study, cortical infarction was induced photochemically in rats by irradiating the intact skull for 20 minutes with green light following the systemic injection of Rose Bengal. Regional cerebral blood flow (rCBF) and local glucose utilization (lCMRglu) were studied autoradiographically at several time periods following irradiation.

Autoradiographic rCBF studies at 30 minutes demonstrated a central ischemic zone surrounded by zones of cortical hyperemia. Asymmetries between hemispheres in brain regions remote from the irradiated zone were apparent at this early time period. For example, rCBF within the frontal cortex was depressed ipsilaterally compared to the contralateral homologous zone. By four hours, the zone of focal ischemia had increased in size and its margins were no longer hyperemic. rCBF was significantly depressed in several ipsilateral cortical brain structures but not in any contralateral brain structures. By five days, rCBF was depressed in both ipsilateral and contralateral cortical areas. Although rCBF was still depressed in some cortical regions at 15 days, significant differences were obtained only within the contralateral auditory and visual cortices.

At 4 hours and 5 days following photochemically induced cortical infarction, lCMRglu was markedly reduced within the lesion center, and irregular regions of moderate-to-marked glucose hypermetabolism were noted within the marginal zone of the developing infarct. Significant differences in glucose utilization were also demonstrated at four hours within ipsilateral brain structures remote from the site of focal injury, including the striatum and hippocampus. By five days, glucose utilization was severely depressed in all ipsilateral cortical areas, but not within any structure contralaterally. This progressive decline in cerebral metabolism followed the earlier progressive decline in cerebral blood flow, and may suggest a cause and effect. It is likely that similar processes are occurring in patients following stroke. Thus the present experimental model should facilitate the understanding of these stroke mechanisms. Supported by NIH grant NS 05820.

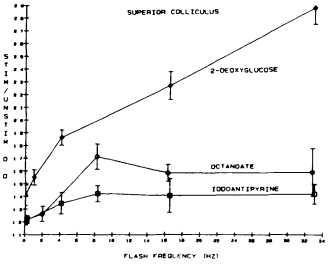
327.8 THERE IS A LOOSE RELATIONSHIP BETWEEN CEREBRAL BLOOD FLOW AND METABOLISM DURING VISUAL STIMULATION. R. Sundermann*, D. Gitelman* A.W. Toga and R.C. Collins. Depts of Psychiatry; Neurology and Neurological Surgery; and the McDonnell Center for the Study of Higher Brain Functions, Wash. U. Sch. of Med., St. Louis, MO 63110

Previous studies (Sokoloff, Fed. Proc. 40:2311, 1981) have indicated a tight relationship between regional blood flow (rCBF) measured with $^{14}$C-iodoantipyrine (IAP) autoradiography and local cerebral glucose utilization (LCGU) measured with $^{14}$C-2-deoxyglucose (2-DG) autoradiography when various grey and white matter structures are compared. As yet no tight coupling between rCBF and LCGU has been demonstrated in response to a range of intensities of physiologic stimulation. We report here the examination of the relationship among 1) graded physiologic visual stimulation, 2) rCBF, 3) LCGU and 4) uptake of $^{14}$C-octanoate (OCTO), a fatty acid metabolized in mitochondria (Rowley & Collins, Brain Research, in press.

Albino rats (300 g) were anesthetized with halothane for placement of catheters and enucleation of one eye. This denervates contralateral visual centers which serve as internal control areas in data analysis. Four hours after halothane, groups of rats were exposed to a flashing checkerboard pattern at different frequencies but will luminal flux held constant for all rats. Quantitative film autoradiography was performed on animals killed at 30 secs for IAP; 2 min for OCTO; and 45 min for DG.

With increasing flash frequency there was a progressive increase in DG labelling in the intact compared to the deafferented superior colliculus (Toga & Collins, J. Com. Neurol. 199:443, 1981). IAP and OCTO labelling increased up to 8Hz but then plateaued. The pattern of the response to graded visual stimulation differed among rCBF, LCGU, and OCTO labelling in the lateral geniculate and visual cortex as well.

The results indicate that the tight relationship between cerebral blood flow and metabolism that is observed when different structures are compared becomes loose when single structures are examined at different intensities of stimulation. Different control mechanisms must exist.




327.9 CEREBRAL BLOOD FLOW AND OXYGEN METABOLISM ARE REGIONALLY UNCOUPLED DURING FOCAL PHYSIOLOGICAL ACTIVATION: A POSITRON EMISSION TOMOGRAPHIC STUDY. Marcus E. Raichle and Peter T. Fox*. (Departments of Neurology and Radiology (Division of Radiation Sciences), Washington University School of Medicine, St. Louis, MO USA 63110).

A large literature has established that cerebral blood flow and metabolic rate are closely coupled to one another throughout the normal resting brain. No studies, however, have directly investigated the coupling of blood flow and metabolism during focal, physiological increases in neuronal activity in normal human subjects.

Regional flow:metabolism coupling during focal brain activation was tested in 9 normal volunteers using $^{15}$-oxygen-labeled compounds ($H_2{}^{15}O$, $O^{15}O$, $C^{15}O$) and positron emission tomography. One resting-state (Set 1) and 3 stimulated-state (Sets 2-4) sets of measurements were obtained during each 2.5-3.0 hr scanning sequence. Focal activation was induced with unilateral vibration of the fingers (D1-5; 130 hz, 2 mm amplitude). Stimulation was begun 0 sec (set 2), 60 sec (set 3) or 300 sec (set 4) before isotope delivery and continued throughout data acquisition (40 - 180 sec).

Discrete, focal increases in rCBF occurred in the postcentral gyrus of the hemisphere contralateral to stimulation in every subject. Stimulus-induced changes in regional cerebral blood flow (rCBF), oxygen metabolic rate ($rCMRO_2$) and the extracted fraction of oxygen (COEF) were calculated within this region as the % $\Delta$ change from the set 1 values.

Regional uncoupling of rCBF and $rCMRO_2$ was found in the zone of focal neuronal activation induced by tactile stimulation. Blood flow increases (mean = 29%) were significantly greater ($p < .000001$, Anov) than metabolic increases (mean = 5%) as measured during identical stimulus conditions, in identical brain regions, in the same subjects. A highly-significant decline in rOEF (mean = -19%) further confirmed that the rCBF increase provided $O_2$ far in excess of the demand from the small $rCMRO_2$ increase. Stimulus duration (Set 2-4)had no significant effect on rCBF, rOEF or $rCMRO_2$. This was not, therefore, a transient overshoot in rCBF nor, conversely, a transient lag in $rCMRO_2$. Uncoupling was highly-focal, being restricted to the zone of rCBF increase. Throughout the remainder of the brain a close correlation (mean r=0.87)between rCBF and $rCMRO_2$ was maintained, across the entire range of rCBF and $rCMRO_2$ values. These observations indicate that the relation of hemodynamics and metabolism is more complex than previously believed.

327.10 **MATURATIONAL CHANGES OF GLUCOSE METABOLIC PATTERNS IN THE HUMAN INFANT DETERMINED BY 18F-2-FLUORODEOXYGLUCOSE POSITRON EMISSION TOMOGRAPHY.** Harry T. Chugani* and Michael E. Phelps (SPON: Jeffrey P. Lieb). Departments of Neurology and Pediatrics, and the Division of Nuclear Medicine and Biophysics, UCLA School of Medicine, Los Angeles, Ca 90024, USA.

The development of positron emission tomography (PET) has made it possible to monitor functional maturational changes in the infant brain through the use of appropriate isotopes. Because normal infants cannot currently be studied with PET for ethical reasons, we have employed a strategy where infants with a diversity of pediatric neurological disorders are studied with PET and 18F-2-fluorodeoxyglucose (FDG). In this manner, developmental changes in local cerebral metabolic rates of glucose (LCMRglc) can be characterized, and at the same time, relevent clinical information obtained to assist in the management of these infants. Our patient population consisted of infants with questionable neonatal seizures, definite neonatal seizures, cerebral embolism from cardiac sources, and otherwise normal infants with facial nevi where the Sturge-Weber syndrome was a consideration. All infants were studied in the awake state, with eyes and ears open, and during continuous EEG recording. In infants between 5 and 26 days of age the glucose metabolic pattern was dramatically distinct from adult patterns, with prominent metabolic activity in the sensorimotor cortex, thalamus, midbrain/brainstem and cerebellum (particularly the vermis). The remainder of the cortex and striatum were relatively hypometabolic. During the second and third months of life, a relative increase in LCMRglc was noted in much of the cortex and striatum, although the frontal cortex and several association cortical regions remained relatively hypometabolic. By 1 year of age, the glucose metabolic pattern clearly resembled that seen in the adult, with prominent frontal and association cortical activity. Our findings are consistent with the known functional organizational changes of the infant brain at 12 weeks of age with respect to EEG activity, sleep-wake patterns and behavior. The gradually increasing cortical glucose metabolism observed in our patients during this period coincides with the suppression of intrinsic subcortical reflexes present in normal newborns. Cognitive or hypothesis-forming development occurs in the human infant towards the end of the first year, and indeed, by this time LCMRglc has increased in frontal and association cortices, areas intimately involved with higher cortical function. The use of PET constitutes a novel approach to the study of the developing human brain, and will undoubtedly also prove to be a powerful tool in which abnormal brain development can be studied.

327.11 THE EFFECT OF DIAZEPAM SEDATION ON CEREBRAL GLUCOSE METABOLISM IN ALZHEIMER'S DISEASE. N.L. Foster*, A. VanDerSpek*, J.C. Sackellares*, M.S. Aldrich*, R.H. Hichwa*, S. Gilman, and B.W. Agranoff. (SPON: M. Bromberg). Depts. of Neurology, Anesthesiology, Internal Medicine and Psychiatry, University of Michigan Medical Center, Ann Arbor, MI 48109.

To examine the effects of mild sedation on glucose utilization in the cerebral cortex, three patients with probable Alzheimer's disease received 5-10 mCi of $^{18}$F-fluorodeoxyglucose (FDG) on two separate days. On one occassion subjects were at rest with eyes patched and ears open, and on the second they received intravenous diazepam titrated to achieve and maintain stage II sleep by clinical and EEG criteria. Rates of glucose utilization were then estimated using positron emission tomography (PET). Cerebral cortex in twelve interleaved horizontal slices in the canthomeatal plane were analyzed using irregular regions of interest. Thus approximately 85% of cortex was measured. Above the level of the cerebellum, cerebral cortex was divided into 4 contiguous regions in each hemisphere, each representing a quarter of the anteroposterior extent of the cortex. Visual cortex was analyzed separately.

PET images showed similar patterns of glucose uptake at rest and with sedation. When normalized to average cortical values, no change in cerebral glucose utilization was seen in any region:

| | % CHANGE WITH MEDICATION (±SD) | |
|---|---|---|
| | LEFT | RIGHT |
| ANTERIOR FRONTAL | 0 ± 1 | 1 ± 3 |
| POSTERIOR FRONTAL | 0 ± 2 | 3 ± 1 |
| ANT TEMPEROPARIETAL | 0 ± 3 | 1 ± 0 |
| POST TEMPEROPARIETAL | 5 ± 1 | 2 ± 4 |
| VISUAL CORTEX | -6 ± 6 | |

Likewise, relative hemispheric cortical metabolism remained unchanged. Two patients who required small doses of diazepam (13.5 and 35.5 mg. over the duration of the study) to achieve sedation showed only minimal (<6%) change in overall glucose utilization, while the third, who received 60.5 mg., had a 49% decrease with medication.

While further study is needed to determine the effect of diazepam sedation on average cerebral cortex metabolism, regional glucose utilization, normalized to overall cortical values, appears to be unchanged by mild diazepam sedation. This technique may permit the study of patients otherwise unable to cooperate with FDG-PET procedures.

(Supported in part by a grant from the W.T. Buchanan Research Fund for the Study of Alzheimer's Disease and Related Dementias).

327.12 A CORRELATIONAL ANALYSIS BETWEEN PSYCHOPHYSICAL PAIN ASSESSMENT, SOMATOSENSORY EVOKED POTENTIALS, AND LOCAL CEREBRAL GLUCOSE UTILIZATION (LCGU) IN NORMAL VOLUNTEERS AND PATIENTS WITH SCHIZOPHRENIA. H.H. Holcomb, W.E. Semple*, M.S. Buchsbaum*, R.M. Cohen*, A.C. King*, J.D. Cappelletti*, L.E. DeLisi*, and L. Flinchbaugh*. Clinical Brain Imaging, LCM, NIMH, Bethesda, MD 20205.

Because patients with schizophrenia differ from normals in their appreciation of pain we have used a (1) nonparametric pain assessment scale, (2) positron emission tomography (PET) of fluorine-18 2-deoxyglucose (F18-2DG) utilization in conjunction with a prolonged, repetitive, noxious, cutaneous, electrical stimulation and (3) a 16 lead somatosensory evoked potential (EP) mapping system to assess the behavioral, neurophysiological and cerebral metabolic response to pain. Normal volunteers (n=23) and drug free schizophrenics (n=19) completed a five minute pain assessment task shortly before receiving thirty minutes of repetitive 1/second electrical shocks to the right wrist. Coincident with the onset of the stimuli ( 2,9,16, and 23 milliamps presented randomly, each lasting one millisecond) 5 millicuries of F18-2DG was administered intravenously. During the stimulation session EP's were collected from 16 left hemispheric scalp leads. Immediately thereafter a second pain assessment was completed and the subject was transferred to the PET scan room where 7 transverse brain images were acquired for LCGU determination. We then generated correlation matrices between pain assessment performance, LCGU, and EP amplitude to determine the relationships between these pain associated behavioral and biological measurements. Both at the left sylvian region near the inferior parietal lobule and the left posterior frontal region, normal subjects exhibit significant correlations between LCGU and pain assessment performance. Those with the best performance exhibit the highest mean glucose metabolic rates. The brains of patients with schizophrenia fail to exhibit such a relationship. The P200 EP amplitude measured by a scalp electrode overlying the inferior parietal lobule is also significantly correlated with psychophysical pain assessment in normal volunteers, but not in schizophrenics. When P200 EP amplitude, averaged over the first 16 minutes of stimulation, is correlated with LCGU, markedly different patterns appear in the two diagnostic groups. Whereas normals exhibit a small number of significant correlations (11) between anatomically appropriate brain regions and their contiguous scalp electrodes, schizophrenics show an unusually large number of significant correlations (111) between many brain regions and all scalp electrodes. These finding suggests that patients with schizophrenia fail to utilize discrete brain regions for specific sensory processing tasks.

## BIOCHEMICAL AND PHARMACOLOGICAL CORRELATES OF DEVELOPMENT II

328.1 LIGHT-DEPENDENT REGULATION OF GABA RECEPTORS DURING DEVELOPMENT. Paul Madtes Jr. National Eye Institute, NIH, Bethesda, MD 20205.

The mechanism by which the development of neurotransmitter systems is regulated is unknown; however, the factors which are involved are being identified. Evidence is accumulating which indicates that the neurotransmitter itself directly regulates the formation of synapses between neurons utilizing that agent in the mature synapse. In particular, the presence of γ-aminobutyric acid (GABA) in the culture media increases the number of neurite-extending cells and the density of neurotubules, rough endoplasmic reticulum, Golgi apparatus, coated vesicles, and other vesicles in cultures of cerebellar granule cells (Hansen et al., 1984). GABA also has the ability to promote synaptogenesis *in vivo* as well as in cultured neuroblastoma cells (Spoerri and Wolff, 1981). Uptake and release of GABA by growth cones isolated from neonatal rat brain suggests that during development this neurotransmitter functions in the regulation of synaptogenesis (Gordon-Weeks et al., 1984). Our laboratory has addressed the developmental interactions among the components of the GABA system in the rabbit retina. When uptake is blocked by *in vivo* treatment with nipecotic acid, thereby increasing the synaptic level of GABA, an increase in the number of receptors occurs (Madtes and Redburn, 1983). GABA agonists in an *in vitro* preparation mimicked this effect, thus confirming the conclusion that this induction involves a direct action at the postsynaptic site (Madtes and Redburn, 1983). Further study demonstrated that the induction is maximal around eye opening, about 10-11 days after birth (Madtes, 1984). We now report that this induction is light-dependent. Pregnant New Zealand white does delivered and reared their pups in total darkness. At selected ages, the pups were sacrificed under red light and the retinas were treated with nipecotic acid. Retinal homogenates were measured for high-affinity $^3$H-muscimol binding. The number of binding sites ($B_{max}$) 9 days and 12 days after birth did not vary as a function of rearing conditions; untreated control tissue from both light- and dark-reared pups had equal $B_{max}$ values. As previously reported, the $B_{max}$ values for control tissues increased 2.5-fold from 9 to 12 days. In contrast, $B_{max}$ values for tissue from the nipecotic acid-treated, light-reared pups were 150% higher than untreated control values at 9 days and 50% higher at 12 days. This induction effect was absent in dark-reared animals. The apparent affinities remained constant under all conditions tested. These results indicate that the presence of light during development is required for the induction of GABA receptors to occur; however, it is not essential for achieving the basal level of receptors which appear during development. These findings suggest that light-dependency is one factor involved in the regulation of the developing retinal GABA system.

328.2 MATURATIONAL CHANGES IN A RETINA-PINEAL GLAND CIRCUIT: SURGICAL AND PHARMACOLOGICAL EFFECTS. L.D. Lytle, D.M. Bronstein*, K.A. Haak*, and G. Torres*. Laboratory of Psychopharmacology, Department of Psychology, University of California, Santa Barbara, CA 93106.

Synthesis of the pineal gland hormone, melatonin, varies diurnally, and is greatest during darkness and lowest during the light phase. Diurnal melatonin synthesis changes with alterations in the activity of N-acetyltransferase (NAT), an enzyme which limits the conversion of pinealocyte serotonin to N-acetylserotonin, an intermediate product which is then O-methylated to form melatonin. In adult rats dark-associated increases in pineal gland melatonin synthesis decline rapidly in animals exposed even briefly to light. These changes in pineal gland NAT activity are controlled by fluctuations in the amount of norepinephrine neurotransmitter molecules released from postganglionic sympathetic nerves onto pinealocyte β-noradrenoceptors. Photic information influences the activity of these noradrenergic nerves via a circuit which begins with the photosensitive cells of the retina, and which includes neurons in the retinohypothalamic tract, suprachiasmatic nuclei, paraventricular hypothalamic nuclei, medial forebrain bundle, intermediolateral nuclei of the spinal cord, and the pre- and postganglionic sympathetic neurons of the superior cervical ganglia (see, for example, D.C. Klein and R.Y. Moore, *Brain Res. 265*:348, 1979).

We studied possible maturational changes in the retina-pineal gland circuit by measuring changes in dark associated pineal gland NAT activity following brief exposure to light in normal, or in surgically or pharmacologically treated developing animals. Different aged (4-8, 16, or 50 days old) male or female albino rats were exposed to fluorescent light (Vita-Lite, DuroTest Corp., NJ; 200 lumens) for a 1 min period during the middle of the dark phase of a 12:12 hr light:dark cycle (lights on at 0700 hr) or were removed from their littermates and mothers but remained in complete darkness for 1 min. Animals were returned to their litters and were killed 30 min later, and pineal glands were assayed radiometrically for changes in NAT activity. In other studies, newborn rats were enucleated surgically or left intact and then were tested for possible light-induced inhibition of NAT activity at 8 days of age, or 3 or 7 day old rats were exposed to light or injected i.p. with the β-noradrenoceptor antagonist drug propranolol 30 min prior to death. Light-induced inhibition of NAT activity was only detected in rats 5 days or older. Light-inhibition of the enzyme activity was not observed in 8 day old blind animals. Light or propranolol inhibited NAT activity in 7 day old rats, but only propranolol reduced the enzyme activity at 3 days of age. Taken together, these data suggest that the retina-pineal gland circuit may become functional as early as 5 days postnatally. However, pinealocyte β-noradrenoceptors (antagonized by propranolol) may reach functional maturation earlier than other components in the circuit mediating lighting effects on the pineal gland. (Supported by grant MH-31134).

328.3 IMMUNOCYTOCHEMICAL LOCALIZATION OF CALCIUM BINDING PROTEIN IN THE PURKINJE CELLS OF THE FROG CEREBELLUM. A.G.Gona, S.Al-Rabiai* and S.Christakos. Univ. Med. & Dent. of New Jersey, New Jersey Med. Sch., Newark, NJ 07103.

In a recent immunocytochemical study on the developing rat cerebellum, Legrand et al (Cell Tiss. Res., 233:389, 1983) found that labelling for vitamin D-dependent calcium binding protein (CaBP) of Mol. Wt. 28,000 dalton was entirely restricted to the Purkinje cells, and that it appeared very early in Purkinje cell development. In view of our interest in Purkinje cell development in the frog cerebellum, we undertook these studies to determine (a) if Purkinje cells of the adult frog cerebellum are immunocytochemically stainable with antiserum prepared against rat renal CaBP, and (b) if the precociously developed Purkinje cells of the frog tadpole cerebellum are similarly stainable. Premetamorphic tadpoles and adults of the bullfrog, Rana catesbeiana, were sacrificed by decapitation and their brains fixed in Bouin's solution. Paraffin sections (8 um thick) of the brains, cut in the sagittal plane, were deparaffinized and sequentially exposed to antiserum to CaBP, sheep antirabbit γ-globulin and PAP, with PBS rinses between steps. The sections were then developed in 3,3'-diaminobenzidine and $H_2O_2$. To verify the specificity of the immunostaining, adjacent sections were treated with antiserum preadsorbed with CaBP, instead of the antiserum. The results showed that both the precociously developed Purkinje cells of the tadpole cerebellum and the mature Purkinje cells of the adult frog are specifically stainable with antiserum prepared against rat renal CaBP. In the region of the auricular lobe, the stained cells included not only precociously developed Purkinje cells, but also cells of smaller size, presumably immature Purkinje cells. It is concluded that the morphologically unique Purkinje neuron of the vertebrate brain acquired the biochemical specialization of CaBP very early during evolution, for some yet unknown function. The presence of immunoreactive CaBP in the smaller neurons and in the Purkinje cells from the larval stage to the adult frog suggests the importance of this calcium binding protein in the Purkinje cell development.

328.4 ADULT OLFACTORY RECEPTOR NEURONS EXPRESS VIMENTIN. D.I. Gottlieb, J.E. Schwob and N.B. Farber*. Dept. Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

During the development of the nervous system, the intermediate filament protein vimentin is found in the proliferating neuroepithelium and neural crest. As development proceeds, postmitotic neurons cease expressing vimentin, and neurofilament proteins begin to accumulate. We have shown that the receptor neurons of the olfactory epithelium deviate from this general pattern in that they continue to express vimentin in the adult rat.

Vimentin-like immunoreactivity was localized in cryostat sections of adult rat tissue using a mouse monoclonal antibody (designated SBV-21) which was generated against bovine vimentin by Dr. Stephen Blose. Vectastain ABC reagents (Vector Laboratories) were used to visualize tissue-bound primary antibody. SBV-21 heavily labels all portions of the primary olfactory projection, including the sensory neuron cell bodies in the olfactory epithelium, the fascicles of the olfactory nerve, and their axonal arbors in the glomeruli of the olfactory bulb. In contrast, anti-neurofilament antisera stain only rare, scattered receptor cells and a small number of axons in the olfactory nerve; anti-GFAP antiserum stains glial processes in the interstices between bundles of olfactory axons.

Direct examamination of the olfactory nerve layer of the olfactory bulb with EM immunohistochemistry shows dense staining of olfactory axons with SBV-21. Some vimentin-like immunoreactivity is also seen in supporting cells in this layer.

The vimentin-like immunoreactive material in the olfactory nerve layer was characterized by SDS-PAGE and immunoblotting. SBV-21 stains only one protein of $M_r$ = 55kD. This band comigrates with vimentin in crude cytoskeletal material from the neonatal rat brain prepared according to the method of Dahl et al. (Eur. J. Cell Biol., 24:191-196, 1981). SBV-21 does not stain neurofilament triplet proteins or GFAP, both of which are also present in these blots.

In conclusion, the vast majority of olfactory receptor neurons and their axons contain vimentin or a protein of identical immunological character and electrophoretic mobility, while identifiable expression of neurofilament proteins is confined to a very small subpopulation. Olfactory receptor neurons are unique among mammalian neurons in that they die and are replaced during adult life normally and following injury. It is interesting to speculate that the expression of vimentin is a marker for the arrest of this neuronal system at an immature state of development.

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328.5 EFFECTS OF POLYAMINES ON GROWTH AND NEURITE FORMATION OF CULTURED RAT CEREBELLAR NEURONS. G.M. Gilad and V.H. Gilad*. The Center for Neuroscience and The Isotope Department, The Weizmann Institute of Science, Rehovot, Israel.

Treatment of newborn rats with exogenous polyamines (PA) leads to increased numbers of sympathetic neurons. In adult early PA treatment accelerates regeneration of crushed nerves. It is unknown whether PA exert their in vivo effects directly, on the neurons themselves, or indirectly. Tissue culture studies may clarify some of these questions. We therefore sought to characterize the effects of PA on growth, survival and neurite formation of dissociated cells (mostly granule neurons) from 6d old rat cerebellum grown under controlled in vitro conditions. At 2d after plating, when 93% of neurons have neurites, ornithine decarboxylase (ODC) activity was still high indicating elevated PA biosynthesis. Addition of 5mM alpha-difluoromethylornithine (DFMO) at plating time, completely inhibited ODC activity and led 24h later to inhibition of cell aggregation with a drastic (90%) inhibition of neurite formation . However, after 48h with DFMO cell aggregation and neurite formation increased and approached the 24h control values. Similar, but more drastic and irreversible effects were observed with 2mM putrescine (put), 0.1mM spermidine (Spd) and 0.1mM spermine (Spm), or with 0.5mM monodansyl cadaverine (DC) an inhibitor of transglutaminase(TG).Washing cultures after 24h and addition of fresh medium led to recovery from the PA effects. Addition of 0.1mM aminoguanidine, an inhibitor of diamine oxidase at plating time prevented the effects of put and spd but not of spm. We conclude: a) inhibition of intracellular PA production delays but does not prevent neuronal growth; b) extracellular PA inhibit cell aggregation and neurite outgrowth; c) the PA effects are due to their oxidative metabolism, and d) TG catalyzed reaction may participate in neuronal growth. - Supported by grants from the Muscular Dystrophy Association and the American Paralysis Association.

328.6 SPECIFIC ALTERATION OF NCAM-MEDIATED CELL ADHESION BY AN ENDONEURAMINIDASE. U. Rutishauser, M. Watanabe*, J. Silver, and E. Vimr*. Dept. of Developmental Genetics and Anatomy, Case Western Reserve Univ. Sch. of Med., Cleveland, Ohio 44106; Dept. of Veterinary Pathobiology, Univ. of Illinois at Urbana-Champaign, Urbana, Illinois 61801

A phage endoneuraminidase that specifically cleaves alpha-2,8-linked polysialic acid has been found to be a useful probe for examining the biological role of this sugar moiety on the neural cell adhesion molecule NCAM. In accordance with other studies, the enzyme caused a 3.3 fold increase in the rate of NCAM-dependent aggregation of membrane vesicles from chicken embryonic brain, but without the nonspecific effects encountered with commercial exoneuraminidases. The enhancement of aggregation was closely correlated with removal of sialic acid as assessed by electrophoretic mobility. Extension of this analysis to cultures of spinal ganglia indicated that removal of sialic acid by the endoneuraminidase results in an increase in the thickness of neurite bundles. This enhancement of fasciculation was reversed by addition of anti-NCAM Fab, suggesting that the enzyme treatment was not toxic and did not cause nonspecific effects on adhesion. Again, the effect of the enzyme was correlated with the removal of sialic acid. Injection of the enzyme into the eyes of 3.5-day chicken embryos produced a striking array of abnormalities in those parts of the neural retina that contain the highest concentrations of NCAM. These perturbations included a dramatic thickening of the neural retina in the posterior pole, a failure of cells in this region to elongate radially as in normal development, formation of an ectopic optic fiber layer along the ventricular margin, and an incomplete attachment of the presumptive pigmented epithelium with the neural retina. These results provide strong evidence that the polysialic acid on NCAM has a direct effect on NCAM-mediated adhesion and that the amount of this carbohydrate is critical for normal morphogenesis of the retina. Supported by NIH Grant HD 18369 and NSF Grant BN82-18700.

328.7 POSTNATAL DEVELOPMENT OF THE SEROTONERGIC INNERVATION OF CEREBRAL NEOCORTEX IN THE RAT. M.E. Blue and M.E. Molliver. Johns Hopkins University School of Medicine, Baltimore, MD 21205.

The 5-HT innervation of neocortex has been characterized in terms of the development of regional and laminar patterns of innervation and of axon morphology. In prenatal stages, the raphe-cortical projection is bilaminar with two sheets of axons, one above and one below the cortical plate, (Lidov & Molliver, BRB 9:559, 1982). The present analysis (employing immunocytochemistry with 5-HT antibody of H. Lidov) shows extensive arborization of 5-HT axons throughout all areas of neocortex during the first postnatal week; distinctive regional patterns of 5-HT innervation are evident in neocortex, and the specific laminar distribution of axons differs from that in the adult. At postnatal day 4 (P4), the fetal innervation pattern is retained with a high density of 5-HT axons in layers I and VI, and in SI there is additional arborization of numerous terminal-like fibers in layers IV and VI; in contrast, there are few axons in layers V or in II+III. There is thus a band of moderate density in layer IV, extending across SI; it does not extend into motor cortex (AGl) which lacks a layer IV. After P6, 5-HT axons arborize extensively in layers II-III and layer V. By day 21, the adult pattern of innervation (Kosofsky & Molliver, in prep) is established in SI: a low density of 5-HT axons in layer IV contrasted with a high density in layers I-III and V-VI. These results lead to the following conclusions: 1) There is a reversal in the laminar innervation pattern during development in SI, since the innervation of layer IV is initially dense and later sparse. 2) The primordial bilaminar ingrowth of 5-HT axons leads to a dual pattern of innervation to the supra- and infra-granular layers, perhaps arising from different sets of raphe cells. 3) Within these two zones, there is a gradient in the sequence of innervation that corresponds to the birthdates and cytologic maturation of cortical neurons, e.g., layer IV is innervated before layers II-III; layer VI before V. 4) Cyto-architectonic differences in innervation are established early in development, at the initial arborization of 5-HT innervation within SI and AGl. To study the morphology of developing axons, the density and spacing of varicosities were quantitatively analyzed in 5-HT axons at different ages. In newborn rats (P4-P10) varicosities along 5-HT axons are far more numerous and considerably more closely spaced than in adults. The average intervaricose distance increases by 300% from P6 to P90. These data suggest that 5-HT neurons form numerous release sites along their axons early in development, and we propose that subsequent axon growth occurs along intervaricose segments with a consequent decrease in synaptic release sites per unit length of axon. (Supported by NIH Grants NS15199, NS21011, NS07396 and by UCP R-340-83.)

328.8 MORPHINE ENHANCES INDEPENDENT FEEDING IN INFANT RATS. C. A. Capuano*, J. Giordano*, D. Bowling* and G. A. Barr* (SPON: G. Gourevitch). Biopsychology Program, Dept. Psychology, Hunter College, City University of New York, N.Y., N.Y. 10021, and Dept. Psychiatry, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

The observation that acute administration of opiate agonists to adult rats increases food intake while opiate antagonists decrease food intake has led to the hypothesis that endogenous opioid systems in part mediate feeding. Although evidence exists that opioid receptors are present in the central nervous system of the rat embryo as early as day 14 postconception, further evidence suggests that the full expression of endogenous opioid systems mediating feeding in the rat is not complete before 14 days postpartum (Aroyewun & Barr, *Neuropharmacol.*, **21**: 757, 1982). Specifically the opiate antagonists naloxone and naltrexone, even at high doses, did not attenuate independent ingestion of milk until 14 days of age.

The present study was undertaken to characterize further the functional ontogeny of opioid systems associated with self-feeding in infant rats. Sated rat pups 3, 5, 7, 14, and 21 days of age were allowed free access to milk that was spread in a thin layer on the floor of the test chamber. Intake was measured following i.p. injection of 0.03, 0.1, 0.3, 1.0 mg/kg of the opiate agonist morphine sulfate, or the saline control. Morphine stimulated milk intake at 3 and 5 days of age within 2 hours of injection, and within 4 hours of injection at 7 days of age and older. Facilitation of intake was delayed at the older ages due to increased sedation. In a final experiment, administration of the opiate antagonist naltrexone HCl (5.0 mg/kg) immediately following morphine injection (0.3 mg/kg) decreased the stimulation of milk intake by morphine to control levels. Naltrexone, by itself, had no effect on feeding. Thus morphine's effects are likely to be mediated by opioid receptors.

The fully functional endogenous opioid system(s) mediating feeding in the adult rat are not fully mature prior to 14 days of age since opiate antagonists are inactive until that age. However, the present results suggest that opioid receptors associated with independent feeding in rat pups are present as early as 3 days postpartum. It is possible that, while the receptors are present and functional early, the ability of deprivation to release endogenous opioids relevant to feeding matures later. This would be consistent with the early appearance of opiate enhancement of feeding and the later appearing inhibition of feeding in deprived pups by opiate antagonists. Finally, the present findings strengthen the argument that the physiological organization of independent feeding in the rat pup, while not fully mature, is continuous with adult feeding and represents the forerunner of later ingestive behavior in the rat.

328.9 CRITICAL DEVELOPMENTAL PERIODS FOR EFFECTS OF ORNITHINE DECARBOXYLASE INHIBITION ON BRAIN DEVELOPMENT AND BEHAVIOR. J.M. Bell, D.S. Madwed*, W.L. Whitmore* and T.A. Slotkin. Dept. of Pharmacology, Duke Univ. Med. Ctr., Durham, NC 27710.

Ornithine decarboxylase (ODC) and its metabolic products, the polyamines, coordinate macromolecule synthesis in developing neural tissues. Inhibition of this enzyme by α-difluoromethyl-ornithine (DFMO) interferes with subsequent cellular replication and maturation. Recent morphological evidence indicates that DFMO is selective for certain brain regions or toward particular phases of cellular differentiation. The present study examined the effects of prenatal vs. postnatal DFMO treatment on brain regional development and sensorimotor behaviors. DFMO was injected into pregnant rats on gestational days 15-17 (prenatal treatment), or into pups from postnatal day 1 through 20. Postnatal DFMO administration did not result in severe body growth reductions, but did cause a profound and progressive deficit in DNA, RNA and protein content in the cerebellum, a region which undergoes major phases of replication and differentiation after birth. Regions having mostly prenatal periods of major replication (cerebral cortex and midbrain + brainstem) also showed minor adverse effects of postnatal DFMO, comparable to those on general body growth. However, inhibition of DNA synthesis ([$^3$H]thymidine incorporation) was detectable in all regions. In contrast, prenatal DFMO treatment resulted in loss of specificity toward cerebellar development and instead exerted a more profound effect on nucleic acids and proteins in cerebral cortex. Thus, DFMO is selective because of regional differences in the timing of replication. DFMO administration also had comparable functional behavioral consequences. Prenatal DFMO administration resulted in severe deficits in sensorimotor development during the immediate postpartum period. The righting, negative geotaxis and pivoting responses were more severely affected in the prenatal relative to the postnatal DFMO treatment group. However, behaviors which develop later were more sensitive to postnatal DFMO; the most severe sensorimotor deficit was in swimming ontogeny, which was profoundly delayed in the postnatal DFMO treatment group. Similarly, the postnatal DFMO group displayed greater deficits in the horizonal and vertical components of an open field test assessed after weaning. These results support the view that the ODC/polyamine system participates in brain development particularly during periods of rapid cellular differentiation. Inhibition of ODC by DFMO during appropriate critical periods results in regional selectivity of effects which produce specific patterns of alterations in sensorimotor development. (Supported by USPHS HD-09713)

329.1 MAMMALIAN CEREBRAL CORTICAL SLICES RESPOND TO VISIBLE LIGHT AT LOW INTENSITIES BY AN ENHANCEMENT OF TRANSMITTER RELEASE. Patricia D. Wade, Jonathan Taylor* & Philip Siekevitz. Rockefeller University, New York, NY 10021.

Intensities of visible light estimated to penetrate the head of the rat enhance release of $^3$H-GABA from cerebral cortical tissue. $^3$H-GABA release experiments. The conditions for $^3$H-GABA release were as in a previous study (Brain Res. 305:259 (1984)). Upon decapitation of male Sprague-Dawley rats, removal of the brain and removal of meninges from the brain, about 0.5mm thick slices were taken from as many regions of cortex as possible. The slices were incubated with $^3$H-GABA in Ringer's for ½ hr. at 36°±0.5°C and then rinsed repeatedly until a baseline level of released radioactivity was established. Then the slices were subjected to elevated $K^+$ (26.4mM) in light or in dark (photographic dark room) conditions. In the light condition, the slices which were in no particular cellular orientation to the light, received white light continuously for 27 min. from a tungsten halogen lamp (Sylvania DWY 650W 120 V). Ultraviolet transmission was prevented by a glass plate. Media were changed and collected every 9 min. and samples from 3 such changes from tissue in either light or dark were counted for radioactivity. Release of transmitter from the tissue in the light was enhanced compared to that in the dark at light intensities of about 0.6mW/cm$^2$ and 1.3mW/cm$^2$. At these two intensities, the average summed and normalized ratios of release in light over release in dark for 27 min. were 1.46±0.13 (std. error of the mean) in 8 expts. and 1.86±0.23 in 4 expts., respectively. Control experiments suggest that the enhancement of release by light is probably not by such non-specific means as: increased temperature, adsorption of GABA to the tissue surface and subsequent release by light or $K^+$, or increased leakiness of the cell membrane. Visible light penetrating the heads of rats. White light was shown on the surface of heads from which the brains had been removed. A photodiode used to measure intensity was placed in the rats' cranial cavities 1 or 2mm central to the dura mater. The percentage of incident light reaching the photodiode within the heads of approximately 8-23 week-old (young adult) rats was 5-12%. These measurements were used with estimates of sunlight intensities to obtain estimates of penetration under natural lighting conditions. We found that the energy necessary for the enhancement of transmitter release can be expected to penetrate a rat's cranial cavity in daylight (sunlight to overcast days) in young adults. An implication from these results is that ambient light may reach the brain in vivo and affect the release of transmitter from cerebral cortex.

Some of the questions still to be answered are: whether released GABA is from neuronal or glial cells, whether other transmitter systems are affected by light, and what wavelengths of light support the GABA release.

329.2 INCREMENT AND DECREMENT SENSITIVITY IN THE LOCUST PHOTORECEPTOR. L.R. Owens and T.E. Cohn. U.C. Berkeley, School of Optometry, Berkeley, CA. 94720.

Responses of locust photoreceptor cells were examined using receiver operating characteristic (ROC) analysis. Stimuli were brief luminance increments and decrements of equal modulation superimposed upon a steady background of low intensity. Under these conditions, fluctuations in the number of quanta are important for they represent an irreducible source of variability which can limit visual sensitivity. Quantum fluctuation predictions were examined by matching experimentally measured ROC curves to predicted curves based on theory (Thibos et al., 1979, Biol. Cybernetics,33:57). Results are based on data from 5 different cells with 100 to 300 stimulus trials each. The test condition involves the presentation of the stimulus plus background versus background alone. Distributions of response with and without stimulus were used to generate an ROC curve. The coordinate points of a poisson ROC fall very near a straight line when plotted with cumulative Gaussian scales for axes. The line is characterized by its intercept with the negative diagonal, called d', and its slope. d' is a measure of the discriminability of the test conditions. The greater d' is, the more discriminable are the 2 conditions. Evidence favoring the quantum fluctuation model would be: d' for decrement stimuli greater than d' for increment stimuli; and ROC slopes greater than unity for decrements and less than unity for increments. For all 5 cells, d' was greater for decrements than for increments, in agreement with the quantum fluctuation model. Mean d' for decrements = 1.86 while d' for increments = 1.24 (S.D.=.14). The slope prediction was not always verified, yet the quantum fluctuation model could not be rejected either. We examined two hypotheses concerning variability intrinsic to the photoreceptor (multiplicative or additive noise) using the models described by Lillywhite, 1981 (Vision Res.21:291). The data presented here fit the additive noise model surprisingly well, while the multiplicative model fit poorly. The additive noise mean value = 98.3 events/10 msec (S.D.=33.2). Perhaps the level of background intensity used here (17 log quanta/sec-sr) is in the range where additive noise begins to have an effect on sensitivity.

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329.3 LOW FREQUENCY VOLTAGE OSCILLATIONS IN HAIR CELLS ISOLATED FROM THE APEX OF THE CHICK COCHLEA. P.A. Fuchs, Dept. of Physiology, Univ.Colorado Health Sciences Center, Denver, CO 80262.

Intracellular voltage recordings from hair cells of turtles, (Crawford and Fettiplace, 1981, J. Physiol. 312:377-412) and frogs (Ashmore, 1983, Nature 304:536-538; Lewis and Hudspeth, 1983, Nature 304:538-541) have revealed that these cells behave as electrical resonators, or filters tuned to unique frequencies. Voltage 'ringing' to injected current steps, and spontaneous voltage oscillations at or near the resonant frequency are signal features of this electrical tuning. In the turtle cochlea each cell's electrical resonance corresponds to, and in large part accounts for, its acoustic frequency selectivity. It is of interest to ask how general such an electrical tuning mechanism might be. Low frequency voltage oscillations were observed in hair cells isolated from the cochlear apex (the presumed low frequency response region) of 15 to 29 day post hatch chicks. All the cells reported on here were obtained from the apical 10% of the cochlea (average 402 μ out of an average total length of 4129 μ). Hair cells in the chick cochlea vary in morphology from tall, inner hair cells nearest the neural insertion, to short outer hair cells. Only (probable) tall cells were chosen for recording (for seven cells the average dimensions were: length, 20.9 ± 0.8μ width, 9.1 ± 0.8 μ and maximum length of ciliary bundle 8.6 ± 0.2 μ, mean ± s.e.m.).

Intracellular recordings from isolated cells were made with the whole-cell suction electrode technique at a temperature of 21-23°C. The resting potential of the cells averaged -73 ± 3 mV. Depolarizing current steps from this level elicited spikelike responses which, though somewhat variable, could be as much as 70 mV in amplitude. When the membrane potential of the cell was raised to -50 mV with steady current, the cell generated repetitive oscillations which occurred at a steady rate in any one cell, and ranged from 4 to 12 Hz. amongst the cells in this study. These oscillations were not purely sinusoidal, but had sharp depolarizing peaks and more prolonged hyperpolarizing troughs, and were 20 to 50 mV in amplitude in different cells. The frequency and amplitude of the oscillations depended on the level of depolarization, being faster (by 1 to 3 Hz.) and smaller (down to 5 mV) at potentials more depolarized than -50 mV. Current steps caused the oscillations to phase-lock such that the average voltage response consisted of a 'ringing' whose frequency varied with the polarity and amplitude of the current step. Thus voltage responses similar to those obtained from electrically tuned hair cells of lower vertebrates also occur in hair cells of the bird cochlea. The underlying ionic mechanism of these voltage oscillations, and their contribution to the normal function of these cells, remain to be determined. Supported in part by grants BRSG-05357 and NS21454 from the NIH.

329.4 VOLTAGE CLAMP ANALYSIS OF MEMBRANE CURRENTS PRESENT IN ISOLATED CONE PHOTORECEPTORS. A.V. Maricq* and J.I. Korenbrot. Dept. of Physiology, University of California, San Francisco, Ca 94143.

Individual cone photoreceptors were obtained by enzymatic dissociation of the isolated retina of the lizard, Sceloporus orcutti. Most of the cells showed a prominent axon and synaptic ending, however only a fraction of them retained their outer segments. Although there was a progressive loss of cells, the photoreceptors could be maintained for several days under tissue culture conditions with little change in their morphology and electrical properties.

Single cone cells were studied in the whole cell clamp mode. Patch electrodes were filled with potassium aspartate with 4 mM ATP, and buffered with Hepes and EGTA. Under current clamp, depolarizing current pulses produced a regenerative voltage response with a peak amplitude of about 25 mv. Hyperpolarizing current pulses produced a distinctive voltage peak reminiscent of the "nose" observed in the photovoltage response to bright lights when recorded with conventional intracellular electrodes. Addition of 5 mM CsCl to the bath selectively eliminated the hyperpolarizing voltage peak, but did not affect the regenerative response to depolarizing pulses.

In voltage clamp experiments with cells held at -60 mv, hyperpolarizing pulses produced a slowly activating inward current which could be entirely abolished by external 5 mM CsCl. Depolarizing pulses above -40 mv resulted in a rapidly activating inward current. Depolarizing pulses above -20 mv produced an additional, slower activating outward current that was apparent first as a decrease in the inward current, and eventually as a net outward current at pulses above 10 mv. This net outward current decayed with a time course of hundreds of ms. The inward current activated by depolarization was studied with the outward current blocked by internal cesium. This inward current was carried by $Ca^{++}$ and by $Ba^{++}$ when $Ba^{++}$ substituted for $Ca^{++}$. External $Co^{++}$ abolished all of the inward current, i.e., there was no evidence that a fraction of the current was carried by $Na^+$. In contrast to some $Ca^{++}$ channels in other cells, where internal fluoride abolishes $Ca^{++}$ currents, we found that the current was preserved when 50 mM CsF was present in the pipette. Comparison of cells with or without axons and synaptic endings revealed a difference in the membrane currents present; $Ca^{++}$ currents were greatly diminished in cells lacking axons indicating a non-homogeneous distribution of $Ca^{++}$ channels over the cell surface.

329.5 RAPID DECLINE IN CYCLIC GMP IN INTACT FROG PHOTORECEPTORS FOLLOWING BRIGHT ILLUMINATION. C. Blazynski, A.I. Cohen, Ophthalmology, Washington University School of Medicine, St. Louis, MO 63110.

Absorption of photons by rhodopsin in rod outer segments initiates a biochemical cascade resulting in the hydrolysis of cyclic GMP. In evaluating the loss of cyclic GMP as a necessary component of transduction, several laboratories have attempted to determine if the onset of the light-induced loss could occur before the known time when rod dark current begins to be suppressed by light. Two laboratories, utilizing intact retinas, and freezing to stop action, reported no loss of cyclic GMP until 2-3 sec after the onset of photopic illumination (Kilbride & Ebrey, 1979; Govardovskii & Berman, 1981). Other laboratories, utilizing portions of photoreceptors, report much more rapid losses of cyclic GMP (Liebman & Pugh, 1979; Woodruff & Bownds, 1979; Cote et al., 1984). We dark-adapted frogs for at least 17 hr and isolated their retinas under infrared illumination. Under infrared, pieces of these retinas, at 22°C, were mounted in an apparatus we devised which first illuminates a piece of dark-adapted frog retina and then propels it so that in 50 msec its photoreceptor surface makes firm contact with a dry, liquid helium cooled copper mirror. Outer segment freezing occurs in a few msec. Several samples were fixed using freeze-substitution, and light microscopy revealed that outer segments were usually folded by the impact to an acute angle with the retinal plane. In a cryostat, two or three 6 μm slices of outer segments were shaved off and assayed. Dark cyclic GMP values were compared with values obtained after multiples of 100 msec of 16000 lux, white illumination. This illumination bleached 4% of rhodopsin in 100 msec. Without exception, the means of 8-10 samples per time point showed a progressive decline in cyclic GMP with time, with values at 250 msec or longer being significantly ($p<.05$) different from the dark level. Dark values of cyclic GMP (mean ± SEM) were 103 ± 5 pmole of cGMP/mg protein. After 100, 200,300,500,1000 and 2000 msec of illumination plus a 50 msec fall to freeze, the values determined were 95 ± 4, 86 ± 3, 79 ± 5, 75 ± 4, 70 ± 4, and 62 ± 2 pmoles cGMP/mg protein, respectively. Our procedure differs from those of previous studies using intact retinas in that all initial manipulations were performed under infrared, our continuous illumination was brighter, outer segments alone were sampled, and freezing was more rapid. Studies with scotopic illuminations are in progress.

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329.6 THE EFFECT OF L-CIS-DILTIAZEM AND C-GMP ON ROD OUTER-SEGMENT CONDUCTANCE. J.H. Stern, U.B. Kaupp & P.R. MacLeish. Laboratory of Neurobiology, Rockefeller University, 1230 York Ave., NY, NY 10021.

Solitary rods were obtained by enzymatically dissociating the retina of the tiger salamander, Ambystoma Tigrinum. The effect of c-GMP and l-diltiazem on the membrane conductance of excised patches and of whole cells was examined. Patches of outer-segment membrane were excised after obtaining gigaohm seals with a suction pipette. The bathing medium consisted of a calcium-free salt solution while the pipette contained the same solution but with 1.8mM $CaCl_2$. Cyclic-GMP reversibly activated a conductance in more than 90% of the patches with a dose-response relationship similar to that found by Fesenko et al.( Nature, 313, 310-313, 1985). The voltage dependence of the c-GMP-induced current was similar to that of the generator current in an intact rod in that it was approximately described by the equation $i=i_0(e^{V/25}-1)$, where i=the c-GMP-induced current, $i_0$=the limiting current for large negative bath potentials, and V=the voltage across the excised patch.

The l-isomer of the calcium-channel blocker, cis-diltiazem, blocked the c-GMP induced current in excised patches. Blockade by l-diltiazem applied to the bathing medium was rapid and reversible. The dose-response relationship for l-diltiazem blockade rose steeply in the micromolar concentration range suggesting a cooperative effect of the channel blocker on membrane conductance. l-diltiazem also affected the shape of the current-voltage curve suggesting that the block was voltage dependent. These effects were specific in that the d-isomer of cis-diltiazem was much less effective as a blocker at comparable concentrations.

Given the action of l-diltiazem on excised patches, it was natural to study its effect on intact cells. We used the intracellular-dialysis (whole-cell) technique to voltage clamp and to introduce substances into cells. In one series of experiments the inclusion of up to 2.3mM l-diltiazem in a one micron (internal diameter) suction pipette did not suppress the light response of dark-adapted, dialyzed rods. Similar results were obtained when dialysis was confirmed by inducing a light-sensitive current in light-adapted cells with concomitant dialysis of c-GMP or 8-Br-c-GMP and l-diltiazem. The absence of block by intracellular l-diltiazem can be explained by one or more of the following: extremely rapid intracellular metabolism, a conductance mechanism inaccessible to the blocker or a difference between the whole-cell and excised-patch conductance mechanisms. Finally, superfusion of dark-adapted rods with salamander salt solution containing 25 to 50 micromolar l-diltiazem but no added calcium ions led to a readily-reversible, partial block of the light response.

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329.7 GUANOSINE 3':5'-CYCLIC MONOPHOSPHATE (cGMP) ACTIVATES A LIGHT-SENSITIVE CURRENT IN AN ISOLATED, DIALYZED ROD OUTER SEGMENT. K.-W. Yau, K. Nakatani* and L.W. Haynes. Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX 77550.

Membrane current was recorded from the outer segment of a toad rod by drawing it partially into a suction pipet containing Ringer's solution[1]. After verifying that the outer segment had a light-sensitive dark current, the inner segment as well as the basal portion of the outer segment outside the pipet were broken off with a probe. This left an open-ended outer segment "envelope" whose interior could be dialyzed with a low Na-high K bath solution. Upon adding cGMP to the bath an inward current appeared at the plasma membrane. Changing bath Ca concentration had no obvious effect on this current. The current increased sigmoidally with cGMP concentration, indicating positive cooperativity of cGMP molecules in turning on the current. Both the Hill coefficient (2.5 ± 0.3 (S.D.)) and the $K_{\frac{1}{2}}$ (52 ± 15 μM (S.D.)) were rather similar to those previously measured from excised membrane patches of rod outer segments[2,3]. Without GTP in the bath the cGMP-dependent current was light-insensitive, but in the presence of 1 mM GTP the current could be completely suppressed by a light flash. With both GTP and ATP present, however, the light-induced suppression of current was more transient and required significantly brighter flashes. The results described here supported the conclusion from excised patch experiments[2,3] that a cGMP-sensitive conductance was present in the plasma membrane of the rod outer segment, and indicated further that this conductance was indeed light-sensitive. The observed effects of GTP and ATP were consistent with present knowledge[4] of the mechanisms controlling light-activated cGMP hydrolysis. From the maximum current inducible by cGMP, we have estimated that ~1% of the light-sensitive conductance is normally open in darkness. The effective free cGMP concentration (present in the intact outer segment in darkness) calculated from this figure and the dose-response relation would be a few μM.

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329.8 VISUAL TRANSDUCTION IN ROD PHOTORECEPTORS FROM TOAD RETINA: EFFECTS OF CYCLIC GMP AND CALCIUM IONS ON CELL-ATTACHED AND CELL-FREE PATCHES OF OUTER SEGMENT MEMBRANE. Gary Matthews, Dept. of Neurobiology & Behavior, SUNY, Stony Brook, NY 11794.

The experiments summarized here examine the roles of calcium (Ca) and cyclic GMP (cGMP) in phototransduction by comparing their effects on light-sensitive channels of intact rods with their effects on cell-free outer segment patches. Recently, a component of dark noise that likely originates in the light-sensitive channels has been identified in cell-attached (1,2) and whole-cell (3,4) patch-recordings from rod photoreceptors. In experiments using patch-pipettes filled with 0 Ca Ringer, I found that the single-channel current estimated from this noise component averaged 18.6 ± 2.0 fA (mean ± s.e.; N=14), which is 5-6 times larger than that reported previously (3,4) in experiments with 1.0 mM external Ca. This suggests that one action of external calcium is to reduce the single-channel current, possibly by occupying the channel but moving through less readily than sodium. The power spectrum of the noise was fitted by a single-Lorentzian, with average corner frequency corresponding to an exponential time-constant of 0.44 ± 0.04 msec. This time-constant gives an estimate for the average open-time of the light-sensitive channels.

Dibutyryl cGMP increased the dark current and the dark noise of cell-attached patches. Noise analysis showed that dibutyryl cGMP increased the frequency of channel-opening in the dark, but did not affect single-channel conductance or open time.

In agreement with previous reports (5,6), the conductance of cell-free outer segment patches was increased by perfusion of the internal face with Ringer containing cGMP. Perfusion with 0 Ca had only non-specific effects. cGMP on the external face was ineffective. The voltage-current relation of the cGMP-dependent current showed outward rectification, as does the light-sensitive current of intact rods. In low external Ca, the cGMP-dependent single-channel current was 20.8 ± 2.2 fA (mean ± s.e.; N=6), similar to that of the light-sensitive channel in low external calcium. With 1.0 mM external Ca, the cGMP-dependent single-channel current fell to about 5 fA, similar to that of light-sensitive channels in intact rods bathed in 1.0 mM external calcium (3,4).

The results suggest that cGMP directly interacts with and opens the light-sensitive channel in cell-free outer segment patches, implying that cGMP is the internal transmitter that keeps the channels open in the dark. Models of the interaction of cGMP with the channel will be discussed. (Supported by NIH Grant EY03821.)

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329.9 ELECTROPHYSIOLOGY OF ISOLATED TASTE CELLS FROM THE MUDPUPPY. S. C. Kinnamon, M. McPheeters, and S. Roper, Rocky Mountain Taste and Smell Center, University of Colorado Health Sciences Center, Denver, CO 80262.

The molecular events underlying taste transduction are unknown. To study chemosensory transduction in detail and elucidate these events, we have developed a technique for isolating individual taste cells from mudpuppy lingual epithelia. Mudpuppy taste cells are large, and when isolated are amenable to both intracellular and single channel recording techniques.

Taste cells were isolated by incubating the lingual epithelium in calcium-free amphibian physiological saline (APS) containing 1% papain, 1% collagenase and 0.25% EGTA for 35 min at room temperature, followed by a wash in APS containing concanavalin A (Con A). With gentle suction, taste buds were removed from the epithelium and individual cells plated on coverslip coated with anti-con A antibody.

Intracellular recordings were obtained from isolated cells and compared with recordings from taste cells in intact epithelia. Taste cells in intact epithelia had high resting potentials (-55 ± 11 mV) and generated action potentials in response to depolarizing current injections; action potentials were typically followed by a calcium-dependent hyperpolarizing after-potential with a duration of several hundred msec. In contrast, isolated cells had somewhat lower resting potentials (-31 ± 15 mV) and failed to elicit active membrane responses to depolarizing current injection, even when hyperpolarized to -80 mV for several minutes before stimulation.

Intracellular recordings were also obtained from taste cells in response to chemosensory stimulation. Taste cells in intact epithelia responded to focally-applied potassium chloride (pressure-ejected from an extracellular micropipette) with an action potential; action potentials were also obtained from most cells in response to citric acid. Subthreshold depolarizing responses were obtained from some cells in response to l-arginine. Isolated cells also responded to chemosensory stimulants: subthreshold depolarizing responses were produced by potassium chloride (all cells) and to l-arginine (some cells). Citric acid elicited subthreshold depolarizations in some cells and hyperpolarizations in other cells.

These data indicate that isolated taste cells retain chemosensory receptors and at least some of the membrane properties characteristic of taste cells in intact epithelium. Thus, it should be possible to examine the molecular events underlying chemosensory transduction in these isolated cells. Supported by grants from Proctor & Gamble, NIH #NS20382, and NIH #AG03340.

329.10 SYNAPSIN I IS PRESENT IN BOTH EFFERENT AND AFFERENT NERVE ENDINGS IN SKELETAL MUSCLE. P.De Camilli[1], M.P.Canevini[1], R.Zanoni[2], M.Vittadello[2], C.Triban[2] and A.Gorio[2]. [1]CNR Center of Cytoph. Via Vanvitelli 32, 20129, Milano, Italy and [2] Fidia Res. Lab. Abano Terme, Italy.

Synapsin I (Syn I) is a major neuron-specific protein, phosphorylated at multiple sites by cAMP-dependent and by Ca/calmodulin-dependent protein kinases. Syn I is concentrated at synapses (being present at virtually all synapses), where it is localized on the presynaptic side. In nerve endings it is associated with the surface of 40-60 nm synaptic vesicles (De Camilli et al., JCB 96, 1337 and 1355 (1983), Huttner et al. JCB 96, 1374 (1983), Navone et al., Science 226, 1209 (1984)). Due to this specific localization Syn I is thought to play an important regulatory role in synaptic vesicle function and, possibly, in neurotransmitter release. We report here that Syn I is also present in at least one type of sensory endings, the spiral endings of muscle spindles.

Frozen sections of fixed skeleteal muscles were double-labeled for Syn I (by immuno-rhodamine) and either for neurofilaments (by immuno-fluoresceine) or for Ach-receptors (by fluoroscein-conjugated $\alpha$-bungarotoxin). Neurofilament stain served as a marker for axons, $\alpha$-bungarotoxin as a marker for motor synapses. Syn I immunoreactivity was found at all efferent axon endings, i.e. at terminals of motor neurons, (including terminals of $\gamma$ fibers) as well as at varicose terminals of autonomic nerves surrounding blood vessels. In addition, sensory endings of the spindles were also highly immunoreactive for Syn I. In both efferent and afferent nerves Syn I immunoreactivity was highly compartmentalized in the most distal portion of the axon, and a rather sharp transition was observed from the neurofilament-rich axon to the Syn I-containing terminal.

Our results indicate the existence of previously unknown functional similarities between afferent and efferent nerve terminals. We do not have, as yet, information on the subcellular localization of Syn I in sensory endings. Small vesicles, similar to vesicles present in efferent terminals, are present in spiral sensory endings. The function of these vesicles is at present unknown. Our result suggest that they might share some important functional properties with synaptic vesicles of efferent endings.

(Supported in part by an MDA Grant to Pietro De Camilli)

329.11 OSMOTIC EXPERIMENTS WITH MUSCLE SPINDLES: ELECTROPHYSIOLOGY AND ULTRASTRUCTURE. D. C. Quick. Dept. of Anatomy, Univ. of Minnesota, Minneapolis, MN 55455.

Muscle spindles were dissected from the cat tenuissiumus muscle and mounted in a perifusion chamber to record static electrophysiological responses (no effort was made in these experiments to dynamically stretch the spindles). In some cases, the capsule was left intact; in others, the capsule was torn open. Experimental solutions were pumped into the chamber, consisting of either a standard Ringer solution, double-osmolar Ringer (Ringer plus 0.3 M sucrose), or half-osmolar Ringer (Ringer minus 0.75 M NaCl). Action potentials in the sensory nerve of the spindle were amplified and recorded continuously on tape for later analysis. After each experiment, a glutaraldehyde solution was pumped into the chamber and the spindle was preserved for electron microscopy.

In standard Ringer, spindles fired at a steady or very slightly falling rate; primary sensory units were more likely to decline slightly than secondaries. In the majority of cases, after introduction of double-osmolar Ringer, the sensory units slowed their firing rate by about 4 to 5% per minute, over a course of 15 minutes or more. Eventually, firing ceased. The effect was reversible - if standard Ringer was reintroduced to the chamber, the firing began to increase by 4 to 5% per minute. Fewer experiments were done with half-osmolar Ringer, but the uniform finding was that firing rate increased. Electron microscopy of spindles fixed under hyperosmolar conditions showed an increased cytoplasmic density of both the intrafusal muscle fibers and the sensory nerve endings. In cases where a dramatic decrease of sensory firing *rate had been observed (but not necessarily a cessation at the* time the spindle was fixed), it was usual to find that the sensory ending had pulled away from the intrafusal muscle fiber at some points. Points of relatively greater adhesion were aligned with the Z- and M-lines of the intrafusal muscle, where desmosomes are often found. In some sections, detachment from the muscle fiber appeared to be nearly complete, even at the Z- and M-lines, but in all cases the sensory endings adhered tenaciously to their basal lamina.

The finding that muscle spindle sensory endings can continue discharging after almost complete detachment from the intrafusal muscle suggests that the endings may respond to gross deformation, rather than deformation at small, discrete points. The basal lamina may be a crucial element in the mechanical linkage between intrafusal muscle fibers and sensory nerve endings.

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330.1 ALTERED AXONAL TRANSPORT OF ACETYLCHOLINESTERASE IN THE SCIATIC NERVE OF ALLOXAN-DIABETIC RATS: EFFECT OF GANGLIOSIDE TREATMENT. A. Gorio, P. Marini*, R. Bianchi*[1] and M. Vitadello. Fidia Research Laboratories, 35031 Abano Terme (PD), Italy, and [1]Institute "Mario Negri", Via Eritrea, 62, Milano, Italy.

The anterograde axonal flow of of AchE was monitored in rats (by the test section method Couraud and Di Giamberardino, J. Neurochem.,35:1053, 1980) five weeks after the injection of alloxan (100 mg/Kg). A 20% reduction of anterograde axonal flow of the enzyme was noticed in diabetic rats compared to the control ones. Sedimentation analysis revealed that such a reduction was mostly due to an impairment of the anterograde transport of $G_1$-$G_2$ molecular forms with a minor, although significant, reduction of $G_4$ form. In contrast the anterograde flow of $A_{12}$ was not affected. Treatment with a mixture of bovine brain ganglioside ($GM_1$, 21%; $GD_{1A}$, 39.7%; $GD_{1B}$, 16% and $GT_{1BT}$, 19% nomenclature according to Svennerholm), starting 7 days after intoxication with alloxan and lasting for 30 days, antagonized the abnormalities of AchE axonal flow. On the other hand no effect was observed in control rats treated with gangliosides, suggesting that gangliosides were effective in improving axonal transport abnormalities induced by diabetic syndrome rather than in activating directly AchE.

330.2 MONOCLONAL ANTIBODIES AGAINST AXONALLY TRANSPORTED PARTICLES FROM RAT SCIATIC NERVE. D.R. Studelska*, T.K. Kimlinger*, J. Katzmann* and S. Brimijoin. Depts. Pharmacology and Laboratory Medicine, Mayo Clinic, Rochester, MN 55905.

Some intracellular materials are transported rapidly (100-400 mm/day) and bidirectionally along nerve axons. Much of this traffic is believed to be associated with vesicles and other membrane-limited organelles. Although microtubules are crucial for rapid axonal transport, recent studies on dissociated axoplasm suggest that the vesicle surface may participate in determining the direction of movement. To explore this possibility we undertook to investigate axonal vesicles by producing monoclonal antibodies against their surface antigens.

Female Balb/c mice were immunized with partially purified vesicles collected from the proximal side of an 18 hr ligation placed on the rat sciatic nerve. The purification was monitored by assays of dopamine-β-hydroxylase (DBH), a membrane-bound enzyme of adrenergic vesicles. The steps in purification were: 1) homogenization and differential centrifugation of desheathed nerve in cold isotonic sucrose; 2) sucrose density gradient ultracentrifugation of the supernatants; 3) Sephacryl S-100 gel filtration of the slow-sedimenting peak of DBH activity; 4) concentration of fractions containing 85 nm DBH-bearing vesicles. The mice initially received s.c. injections of vesicles in complete Freund's adjuvant. Immunization was repeated a month later by i.p. injections without adjuvant and again 3 days before the spleen cells were isolated for fusion with FO (first fusion) or NS1 (second fusion) myeloma cells. Media from primary fusion cultures were assayed with an ELISA. Freshly prepared sucrose gradient fractions containing the slow sedimenting DBH vesicles were bound to microtiter plates at alkaline pH and incubated with culture supernatants overnight at 4°C. The plates were then washed and exposed for 2 hr to alkaline phosphatase-conjugated goat anti-mouse IgG, after which binding was detected colorimetrically. Positive primary cultures were cloned by one or two subcultures at limiting dilution. The resulting clones were reassayed twice with the ELISA before expansion as ascites tumors in pristane-treated mice.

The first fusion produced 18 positive clones from 5 different primary cultures. Different patterns of immunoreactivity with sucrose gradient fractions of nerve extracts showed that some of the clones isolated from the same primary cultures produced different antibodies. A second fusion produced 61 positive clones from 18 different primary cultures. Several of these antibodies may recognize slowly transported or stationary antigens (e.g., of the cytoskeleton and axolemma). However most of the antibodies tested to date are directed against materials that accumulate rapidly with ligation. In addition, some appear to recognize antigens that are transported in only one direction along the axon. (Supported by NIH grant NS 11855.)

330.3 TRANS-SYNAPTIC TRANSFER OF WHEAT GERM AGGLUTININ-HORSERADISH PEROXIDASE (WGA-HRP) IN THE NERVOUS SYSTEM. M. El-Kalliny*, R.D. Broadwell, and B.J. Balin* (SPON: T. Rogers). Divs. of Neurosurg. and Neuropath., Univ. of MD, Balto., MD 21201.

The transfer of endogenous macromolecules (proteins, peptides) between neurons is very likely a consequence of the secretory process (i.e., synthesis, packaging, storage, and release of the molecule). The Golgi complex occupies a nodal point in the scheme of the secretory process. This organelle within cells in general is responsible for the packaging of molecules destined for export and exocytosis. WGA, a plant lectin, conjugated to HRP is an ideal tracer molecule with which to investigate the neuronal secretory process. Unlike native HRP and specific ligands that enter cells by fluid phase and receptor mediated endocytoses and become sequestered in endosomes (prelysosomes) and dense bodies (lysosomes), WGA-HRP is taken into cells by adsorptive endocytosis and is localized within the innermost (trans) Golgi saccule and lysosomes. Our in vivo studies indicate that WGA-HRP is endocytosed by cell bodies, dendrites, and axon terminals. Internalized cell surface membrane tagged with WGA-HRP is directed to endosomes with subsequent transfer of the tracer to the trans Golgi saccule; the tracer then is packaged into Golgi-derived vesicles (40-70 nm)/vacuoles (80-100 nm) and secretory granules (100-200 nm), depending upon the type of neuron. A population of these Golgi-derived, peroxidase-positive structures likely represents primary lysosomes that fuse with endosomes and secondary lysosomes; another may be destined to replenish cell surface membrane in the dendrites, perikaryon, and axon; and a third population undergoes transport by axoplasmic flow to the axon terminal for exocytosis. This transport is independent of the endoplasmic reticulum, which is not peroxidase-positive. Native HRP fails to label Golgi saccules in the neuron and consequently is not transported in Golgi-derived products; however, anterogradely transported tubules and dense bodies are peroxidase-positive in neurons exposed to native HRP and WGA-HRP. These structures may arise from perikaryal lysosomes. Exocytosis and anterograde trans-synaptic transfer of WGA-HRP, but not of native HRP, does occur. Peroxidase-positive organelles identified in post-synaptic neurons include endocytic vesicles and tubules, endosomes, dense bodies, and less frequently the trans Golgi saccule. WGA-HRP and/or fragments thereof conceivably enter post-synaptic cells by fluid phase and adsorptive endocytoses. The potential for macromolecules (excluding virus) in neurons to be delivered across the synapse, whether in the anterograde or retrograde direction, is determined in large measure by the secretory process and involvement of the Golgi complex. NINCDS Grant #NS18030.

330.4 INTRACELLULAR TRAFFICKING OF TRANSPORTED GLYCOCONJUGATES IN DENDRITIC COMPARTMENTS OF OLFACTORY BULB NEURONS. John G. Wood* and John W. Scott (Spon: T.A. Harrison), Department of Anatomy, Emory University School of Medicine, Atlanta, Georgia 30322.

Although considerable information is available defining the intracellular route taken by macromolecules undergoing transport (primarily retrograde) in axons, there is as yet little equivalent information for dendritic transport. The linear orientation of mitral cell basal dendrites makes these cells ideally suited for such studies. WGA-peroxidase conjugates were iontophoretically injected in a site restricted to the deepest one-third of the external plexiform layer in the rat olfactory bulb. After 3 and 24 hrs, the animals were sacrificed by vascular perfusion of fixatives, and 80 µm vibratome slices of bulb tissue were processed for peroxidase visualization and examination at the light and electron microscopic levels. At the light microscopic level labeled mitral cell soma were observed with labeled basal dendrites both proximal and distal to the injection site. The dendrites and somata were lightly labeled, often with a filamentous appearance, and with frequent darker bodies which were larger in the proximal dendrite. At the electron microscopic level, large labeled processes were confirmed as mitral cell dendrites that were frequently observed to be in synaptic contact (dendro-dendritic) with granule cell dendrites. In dendrites between the soma and the injection site, the label was associated with membranous profiles resembling classical endocytotic vesicles. In those labeled dendrites emanating from a cell soma on the side distal to the injection, the intracellular localization of reaction product was invariably restricted to tubulo-vesicular profiles located primarily subadjacent to the dendrolemma. In these same profiles an irregular pattern of strips of extracellular labeling was observed, which was concentrated in the synaptic cleft at sites of reciprocal dendro-dendritic contacts between mitral cell and granule cell dendrites. The results suggest that WGA-peroxidase undergoing dendritic transport in mitral cells escapes degradation in the soma, and may subsequently be preferentally delivered to synaptic sites.

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330.5 **A METHOD FOR THE STUDY OF AXONAL TRANSPORT BY DIRECT VISUALIZATION IN INDIVIDUAL MAMMALIAN AXONS IN-VIVO.**
T. Spagnolia, R. Rumpf, W. J. Levy, and D. H. York. University of Missouri Health Sciences Center, Columbia, Missouri 65212

For the direct visualization of axon transport in-vivo, we pass the sciatic nerve of a rat through a perfusion chamber designed for controlled temperature and flow. The rat is anesthetized with Na Pentobarbital and has had a previous dissection of sciatic nerve throughout its course distal to the gastrocnemius. Platinum stimulating electrodes are placed approximately at the sciatic notch and silver recording electrodes are placed distal to the chamber, as are EMG recording electrodes in the intrinsic foot muscles. The chamber itself sits on an AVEC DIC type microscope with rectified Normaski optics. This is a Nikon optiphot using a Dage MTI vidicon tube and control box. A Panasonic 800 line monitor and a Pansonic 500 line 3/4 inch VTR are used for image viewing and storage. The DIC optics use a 100x oil immersion objective (NA1.25) and condenser (NA 1.3). The microscope stage has been custom machined to allow placement of the rats, such that the sciatic nerve passes through the chamber bewteen the objective and the condenser. Temperature is maintained by a control heating environment. The chamber itself is a 25 mm diameter chamber with a coverslip bottom. Ports for in-flow and out-flow of solution connected to an 10-roller peristaltic pump, with temperature monitoring and a removable coverslip top which sits on a silicon elastomer spacer, 100 microns thick. The nerve is fed through the chamber in which Dulbecco's solution with the addition of 5 millimolar ATP and a carboxygen bubbling. An enzymatic dissociation regiment is used consisting of collagenase, trypsin and hyaluronidase which are passed through in seqeuence at 20 minute intervals with minimal agitation. This dissociates the extracellular matrix external to the Schwann cells. Continuous electrical monitoring shows that there is preservation of the nerve action potential and EMG. Examination of the axons under both phase contrast and rectified Normaski light microscopy, as well as osmic acid staining, shows no gross alteration of morphology in the vast majority of axons, compared to the predissociation state. There is some widening of the Schmitt-Lanterman clefts and nodes of Ranvier. Once the axons are dissociated, 50µ microscissors are used to pare a sufficient number of axons away from the nerve so that a single layer remains on the bottom. A coverslip is then advanced on top of these by a threaded ring on the chamber to narrow the intercoverslip distance and produce good optical visualization. Direct observation of axon transport in individual axons is then possible. The axons are in continuity with the spinal cord neuron proximally and the muscle and target organ distally. Electrical stimulation can be carried out during this observation period, using the proximal electrodes. Axon transport was observed with particle movement in both directions, predominantly retrograde. The fast rate is consistent with previous reports of approximately 400 mm per day. This model should provide opportunities to study axon transport and axon function in-vivo in animals of various ages and with various disease processes. Chamber manipulations or manipulations of the animal, which would involve the central neuron and target organ, can be carried out separately.

330.6 TRANSLOCATOR PROTEIN (KINESIN) FOR RAPID AXONAL TRANSPORT MEDIATES ANTEROGRADE MOVEMENT OF BEADS ALONG PURIFIED MICROTUBULES. B.J.Schnapp, R.D.Vale*, T.Mitchison*, T.S.Reese and M.P.Sheetz*. Lab or Neurobiology, NINCDS, NIH, at the Marine Biological Lab., Woods Hole, Mass. 02543.

A soluble factor in axoplasm from the squid giant axon attaches to organelles or carboxylated latex beads and promotes their translocation along purified microtubules (Vale et al, Cell, 40:559, 1985). This factor, which has now been purified from squid optic lobes and bovine brain (Vale et al,Cell,in press) is a protein complex having an apparent molecular weight of approximately 600 Kd and is called kinesin. When kinesin is adsorbed to latex beads and the beads added to microtubules purified from squid optic lobes, video microscopy shows that single microtubules have beads moving along them in one direction only. Microtubules in axons are all oriented in the same direction with their plus ends toward the nerve terminals (Burton and Paige, PNAS, 78:3269, 1981). To determine the polarity of bead movement, microtubules were polymerized from purified bovine brain tubulin by nucleation from centrosomes isolated from a neruoblastoma cell line (Mitchison and Kirschner, Nature, 312:237, 1984). Microtubules polymerized off centrosomes are oriented with their plus ends free and their minus ends toward the basal bodies. When the movement of kinesin-coated beads was assayed on these centrosomal microtubules, all bead movement occured from the minus to the plus ends, corresponding to anterograde transport in the intact axon.

Single microtubules dissociated from the extruded axoplasm of the squid giant axon support the movement of vesicular organelles in both directions (Schnapp et al, Cell, 40:455, 1985). Bidirectional movement of both beads and organelles along centrosomal microtubules is also observed in the presence of a crude supernatant fraction from squid axoplasm. We are using the centrosomal bead movement system as a polarity assay to dissect out those components in the squid axoplasm which determine the direction of organelle movement.

330.7 REGIONAL DIFFERENCES IN MICROTUBULE-POLARITY RATIOS CORRESPOND TO THE DIRECTION OF AXOPLASMIC TRANSPORT IN LOBSTER AXONS. T.A. Viancour, D.S. Forman and D.L. Shevitz*. Biol. Sci., Univ. Maryland Baltimore County, Catonsville, MD 21228, and U.S.U.H.S., Sch. Med., Anatomy, Bethesda, MD 20814-4799.

We have investigated the relationship between microtubule polarity orientation and the direction of axoplasmic transport and found a significant correspondence between the two. The investigation was suggested by the findings of Forman et al (J. Neurosci., 3, 1279-1288, 1983), which reported a regional difference in the predominant direction of axoplasmic transport of particles and mitochondria in 'fast opener' and 'closer' motor axons in the walking legs of lobster (Homarus americanus). In those axons, anterograde transport predominates in a thin, less than 1µm thick, circumferential rind of axoplasm adjacent to axonal membranes, and retrograde transport predominates throughout the rest of the axoplasm.

Microtubule polarities in the identifiable 'opener' axons were determined by the directions of curvature of protofilament hooks which were formed on existing microtubules by incubation in a PIPES buffered solution containing bovine microtubule protein (see Heidemann and Euteneuer in Methods in Cell Biology, v24, pp207-216, Acad. Press, 1982).

Within 0.5 µm of the axonal membranes, roughly half the microtubules have positive ends oriented toward axonal cell bodies and half have the opposite orientation. Throughout the remaining axoplasm nearly all of the microtubules are oriented with positive ends oriented away from axonal somata. There appears to be an abrupt transition from the axolemmal to the lumenal polarity distribution and the polarity ratios of the two regions are significantly different. Preliminary observations suggest that axolemmal microtubules of the same polarity may be clustered.

The regional difference in microtubule orientation is therefore identical to the regional difference in the predominant direction of particle movement in these axons. This correspondence indicates an important relationship between transport direction and microtubule polarity. Furthermore, these findings indicate that microtubule organizing centers may be located within distal axonal segments.

330.8 ACTIVE SINUSOIDAL SHORTENING OF AMPUTATED AXONS SHARES CHARACTERISTICS WITH NORMAL AXON GROWTH.
B.F.Schneider*, E.B.George*, M.J.Katz, and R.J.Lasek (SPON: S.T.Brady). Neurobiology Center, Case Western Reserve University, Cleveland, OH 44106

When axons are amputated from their cell bodies in culture, they undergo an energy dependent process of sinusoidal shortening. We found that this process spontaneously occurred in some intact axons and that it never occurred in transected processes of non-neuronal cells. Because, this suggests that active sinusoidal shortening may be a natural process peculiar to axons, we compared it to the process of normal axonal growth.

Dissociated neuronal cultures were prepared from embryonic (7-12 day old) chick dorsal root ganglia. After 16-24 hours in culture, axons were transected using a glass microneedle. The sinusoidal shortening process required both glucose and pyruvate in the medium, and could be inhibited by either iodoacetate (2.5 mM) or potassium fluoride (15 mM). Thus, the shortening apears to depend on active glycolytic metabolism.

Measurements of sinusoids in shortening axons were made sequentially after amputation, using time-lapse videomicroscopy. 118 measurements on 42 axons were used to calculate the rate of shortening. The average rate of shortening was 1.25 ± 0.7 um/min (1.8 mm/day). The fastest rates (99th percentile) observed for sinusoidal shortening ranged between 5.5 um/min (7.9mm/day) and 6.25 um/min (9.4 mm/day). These rates are similar to the calculated maximum rate of 6.5 um/min (9.3 mm/day) for axonal elongation in the same type of cultures (Katz et al, Cell Motility 4:351, 1984). We interpret these maximum rates as representing the optimal intrinsic rates of these processes. These rates are all comparable to the rate of slow transport in developing axons (5-10 mm/day).

The similar metabolic requirements and the similar rates of change in axon length suggest that the processes of sinusoidal shortening and normal axonal growth share similar underlying mechanisms.

330.9 MECHANISM OF CYTOPLASMIC STREAMING IN GIANT CHARACEAN ALGAE CELLS: POSSIBLE RELATIONSHIP TO SLOW AXONAL TRANSPORT. R. Kachar* and T.S. Reese. (SPON: M.Dubois-Dalcq). Laboratory of Neurobiology, NINCDS, NIH, Bethesda, MD 20205.

Evidence has accumulated that cytoplasmic streaming in giant characean algae cells depends on an actomyosin-driven shearing force generated along stationary actin filament cables at the cell cortex (Kamiya, Ann. Rev. Plant Physiol. 32:205, 1981). The binding and continuous unidirectional movement of organelles along actin cables can be visualized directly by video microscopy after dissociation of the cytoplasm of the algae Chara (Kachar, Science, 227:1355, 1985). Individual organelles move at mean rates of either 11.2±0.1 or 62.1±1.1 μm/sec. Freeze-fracture electron microscopy after direct freezing demonstrates that the organelles moving at 62 μm/sec, which is also the rate of cytoplasmic streaming in intact cells, correspond to vesicular and tubular fragments of endoplasmic reticulum. In fast frozen intact cells, examined by freeze-fracture or freeze-substitution, the endoplasmic reticulum is a tridimensional network of continuous tubular and cisternal structures which is integrated, along with the other organelles, into the cytomatrix to form a cohesive cytoplasm. It is likely that the streaming of the whole cytoplasm depends on the shearing force generated along stationary cortical actin cables by myosin or a myosin-like ATPase at the surface of the endoplasmic reticulum (see Williamson, Eur. J. Cell Biol. 20:177, 1979); the continuous endoplasmic reticulum network in these cells provides the effective means of mobilizing cytoplasm distant from the cortical actin filament bundles, where the motive force is generated (Bradley, J. Cell Sci. 12:327, 1973).

The movement of neurofilament bundles and other cytoplasmic elements down the axon, though several orders of magnitude slower than the cytoplasmic streaming in Chara, might depend on a similar mechanism. Segments of the smooth axoplasmic reticulum typically lie near the axolemma (Rambourg & Droz, J. Neurochem. 35:16, 1980).This continuous, extensively branching axonal reticulum is the only membranous organelle that regularly intrudes into the neurofilament bundles (Schnapp & Reese, J. Cell Biol. 94:667, 1982). Furthermore, individual actin filaments, though not actin cables, are present in the subaxolemmal region (Metuzals & Tasaki, J. Cell Biol. 78:597, 1978; Hirokawa, J. Cell Biol. 94:129, 1982). It is not known whether myosin is associated with the subaxolemmal endoplasmic reticulum, but it is clear that anatomical relationships comparable to those postulated to power cytoplasmic streaming in Chara are present in the axon. The lower rate of slow axonal transport need not rule out a myosin-based system because these systems are known to vary widely in their intrinsic rates (Sheetz et al., J. Cell Biol. 99:1867, 1984).

330.10 REDISTRIBUTION OF ACTIN ALONG WITH MICROTUBULES AND THEIR ASSOCIATED COUPLERS IN RAT SCIATIC NERVE AXONS FOLLOWING ADMINISTRATION OF B,B'-IMINODIPROPIONITRILE (IDPN). R.G. Nagele*, S. Wang*, M.C. Kosciuk* and H. Lee*. Univ. of Med. and Dent. of New Jersey-Sch. of Osteopathic Med. and Rutgers Univ., Camden, NJ 08103.

To date, the most compelling evidence for the direct participation of microtubules (MTs) in the fast axonal transport of membrane bound organelles (MBOs) has emerged from studies on the toxic axonopathies induced by IDPN in rodents. IDPN induces a reorganization of the axonal cytoskeleton in which MTs aggregate into a single cluster and neurofilaments (NFs) are displaced toward the periphery of the axon (Papasozomenos et al., J. Cell Biol., 95:672,1982: Griffin et al., J. Neurosci., 3:557, 1983). This cytoskeletal reorganization has no effect on fast axonal transport which occurs exclusively within MT clusters. In the present study, we have used IDPN-treated rat sciatic nerve axons as a model system to test the following hypotheses: (1) If microtubule-neurofilament couplers, which link these cytoskeletal elements in a variety of neurons(Nagele, R.G. and Roisen, F.J., Brain Res., 253: 31, 1982), are involved in the fast axonal transport of MBOs, then they should be redistributed in IDPN-treated axons so as to remain associated with MTs and MBOs. (2) If the fast axonal transport mechanism is based on an actin-myosin contractile system associated with MTs, then these motility-related proteins should colocalize with MTs and become concentrated within IDPN-induced MT clusters.

Sciatic nerves on each side of adult male rats were injected subepineurally with 5 μl of IDPN (diluted 1:1 in saline). Controls were injected with an equal volume of saline. Animals were sacrificed at intervals and segments of sciatic nerve containing the injection site were fixed for electron microscopy or prepared for the localization of actin using indirect immunofluorescence.

Results show that adjacent MTs within individual MT clusters are separated by a rather uniform distance. Measurements of inter-MT spacing revealed a clear tendency for MT clusters to become more compact during the first 12 hours postinjection. Couplers remained associated with MTs and MBOs during their segregation from NFs and were not found in peripheral regions of axons that contained only NFs. This finding strongly suggests that MTs are the "parent" structure to which couplers are attached and further supports an important role for couplers in fast axonal transport. Indirect immunofluorescence revealed that actin, like tubulin, is concentrated within MT clusters in IDPN-treated axons. This colocalization of actin and MTs along with knowledge that fast axonal transport occurs exclusively within MT clusters of IDPN-treated axons adds strong support to the notion that at least part of the molecular mechanism for fast axonal transport involves an actin-myosin contractile mechanism.

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330.11 AXONAL TRANSPORT OF CLATHRIN-ASSOCIATED PROTEINS. D. J. Gower* and M. Tytell†, Depts. of Surgery, Section on Neurosurgery* and Anatomy†, Wake Forest Univ. Med. Sch., Winston-Salem, NC 27103.

Clathrin is the primary protein that makes up the lattice-like structure which forms the "basket" surrounding a coated vesicle. The closure of the basket is postulated to depend upon 2 proteins of 30-36 k daltons, known as the clathrin associated proteins (CAPs). Clathrin is known to be axonally transported in association with the proteins of slow component b (Garner and Lasek, JCB 88:172, 1981), but it has not been determined whether the CAPS are transported with it. We have investigated this question because the result will provide some insight into the state of axonal clathrin.

A unilateral intraocular injection of 0.5 mCi of $^{35}S$ methionine was administered to nine adult male Hartley guinea pigs under anesthesia. Groups of three animals were sacrificed at 6 hours, 5 days and 30 days, to label the proteins of fast component (FC) and slow components b and a (SCb & SCa), respectively. The ipsilateral optic nerve was removed from each animal, as well as the contralateral optic tract in the 30-day animals. The tissue was homogenized in "clathrin buffer" (100 mM MES pH 6.5, 1 mM EGTA, 0.5 mM $MgCl_2$, 0.02% sodium azide, 7 mM β-mercaptoethanol and centrifuged at 20,000 x g for 30 minutes. The supernate was TCA precipitated and the protein redissolved in lysis buffer (9M urea, 5% BME, 5% ampholines, pH 4-9, 8% Triton X-100, pH adjusted to 7.5). A stock of partially purified clathrin plus CAPs was prepared from guinea pig brain by a modification of the method described by Shook and Puszkin (Methods of Enzymology, vol. 98:30, 1983). The purified clathrin and each of the four specimens from optic nerve and optic tract were comigrated on two dimensional SDS-polyacrylamide gels and analyzed by fluorography.

The CAPs were found to be primarily transported with the proteins of SCb. These data imply that axonal clathrin is transported in a form that may be competent to polymerize into the coated vesicle lattice. In this light, the paucity of coated vesicles within the axon suggests that, in addition to the presence of CAPs, the clathrin may require a distinctive intracellular environment to form baskets. Those conditions probably exist only at synaptic terminals and in the soma. (Supported by Grants from Southern Med. Assoc. to DJG, NC United Way and Bowman Gray Med. School to MT & DJG.)

330.12 INTRACELLULAR DIFFUSION OF $CA^{++}$ AND THE FAST AXONAL TRANSPORT. D.C. Chang, Physiol. Dept., Baylor College of Medicine, Houston, TX 77030.

It is evident that in the cytoplasm some ions (such as $Na^+$ and $Ca^{++}$) are bound or sequestered into organelles. Ealier studies suggest that $Ca^{++}$ ions inside the nerve cell are transported by a facilitated mechanism. We injected radioisotope labelled $Ca^{++}$ ions into the giant axon of squid to investigate whether the movement of these ions is coupled with the mechanism of fast axonal transport.

The spatial distribution profile of the labelled ion along the giant axon was determined at different times after the intracellular injection. The axon was rapidly frozen in liquid nitrogen and cut into small segments while still frozen. The radioactivity of each segment was then counted by a scintillation counter. We found no evidence that the distribution of $Ca^{++}$ is affected to any significant extent by the fast axonal transport. First, the ionic distribution curve was almost symmetrical. Second, the peak of the ionic profile coincided with the location where the labelled ions were injected. If the ions were carried by the fast axonal transport, there would be a shift of the ionic profile toward the direction in which the ion-sequestering vesicles moved.

For the purpose of comparison, we have also studied the intracellular transport of radioactive $Na^+$. The distribution profile of $Ca^{++}$ is considerably narrower than that of the $Na^+$ indicating that the effective diffusion of $Ca^{++}$ is much slower than $Na^+$. But even in the case of $Na^+$, the effective diffusion within the axoplasm was significantly slower than that of a free solution. This result is different from the finding in the earlier study of $K^+$ transport within the giant axon of sepia. The implication between the effective diffusion coefficient and the activity of free ions in the axoplasm will be discussed. (Work partially supported by an ONR contract.)

330.13 BEADING OF NERVE FIBERS AND FAST AXOPLASMIC TRANSPORT. S. Ochs and Jersild, R., Jr., Depts. of Physiology/Biophysics and Anatomy, Indiana University School of Medicine, 635 Barnhill Drive, Indianapolis, IN 46223.

In one theory of axoplasmic transport, fluid channels in a structured network within the axoplasm are assumed to be the path by which various components are transported in nerve fibers. To test that concept nerves were stretched to cause the fibers to become beaded. Beading appears as a series of constrictions 10-25 um in length at intervals of 20-50 um along the fibers, collapsing fluid channels and possibly blocking transport. Cat L7 dorsal root ganglia were injected with $^3$H-leucine and after 2 hr of downflow of labeled proteins *in vivo*, the sciatic nerves were removed and placed in a chamber with a weight of 1-35 gm tied to the distal end of the peroneal branch to initiate and maintain beading. The tibial branch remained unstretched. The *in vitro* preparation was kept at 38°C and oxygenated with 95% $O_2$ + 5% $CO_2$ for times up to 5 hr after which a portion of the peroneal nerve was frozen *in situ* for freeze-substitution while still under stretch. Freeze-substitution is required to hold the labile shape of the beaded fibers during histological preparation. Fibers in the unstretched tibial nerves were mostly cylindrical while those in the stretched peroneal nerves showed the series of constrictions along their fibers typical of beading. In cross-section the axonal area in the constrictions was reduced to <5% of normal. A close packing of microtubules and neurofilaments in the constrictions was observed in slam-freeze preparations examined by EM. Beading did not, however, cause a block of transport as shown by the normal outflow pattern of labeled proteins. The fronts were, however, a little retarded in the stretched peroneal nerves compared to the unstretched tibial nerves and had somewhat less labeled material in the crests. Considering the marked reduction of axonal cross-sectional area with little evidence of fluid channels remaining, and the possibly greater viscosity which may be present if beads are maintained for the whole time of downflow, this would appear to require an active propulsion through these regions of resistance. Such is envisioned in the transport filament hypothesis where ATP is utilized to drive carriers, to which various transported components are bound, against viscous resistance. Supported by NIH PHS ROI NS 8706-15 and NSF BNS 82-17727.

330.14 ARE ELEVATED LEVELS OF FAST TRANSPORT ESSENTIAL FOR OUTGROWTH OF GOLDFISH OPTIC AXONS? Janet R. Sparrow and Bernice Grafstein. Dept. of Physiology, Cornell University Medical College, New York, NY 10021

Although many regenerating neurons do not exhibit changes in axonal transport during regeneration, goldfish retinal ganglion cells show large increases in axonal transport during regeneration. For instance, the amount of fast-transported protein, which comprises organelle constituents, including plasmalemma, is increased as early as one day following axotomy, and by 2 weeks postoperatively the amount is 3-5 fold greater than normal (Giulian et al., 1980, J Biol Chem 255: 6494; McQuarrie and Grafstein, 1982, Brain Res 235: 213). We have therefore investigated whether this increase in fast transport is necessary for sustained outgrowth of the axons.

Following optic nerve crush, intraocular injection of the drug monensin (Hammerschlag et al., 1982, J Cell Biol 93: 568) reduced fast transport of $^3$H-proline-labelled protein in the regenerating axons to the level normally present in unlesioned axons. During the first week of regeneration, outgrowth proceeded at a nearly normal rate; between 8 and 11 days, however, the axons advanced very little. The effects of diminished fast transport on the regeneration of these axons was not limited to the early stages following axotomy: administration of monensin beginning at the time that the axons arrive in the optic tectum delayed visual recovery.

We also sought to determine whether the elevated levels of fast transport occurring after a single axotomy are sufficient to support regeneration at an enhanced rate. Outgrowth of the optic axons was accelerated by subjecting the axons to two lesions separated by an interval of 2 weeks (the "conditioning lesion" paradigm (McQuarrie and Grafstein, 1981, Brain Res 216: 253)). Since the amount of fast-transported protein in conditioned nerves is markedly greater than in nerves regenerating after only one lesion (McQuarrie and Grafstein, 1982, Brain Res 251: 25), we administered monensin on the day preceding and the day after the second lesion, which reduced fast-transport in the conditioned nerves to the level present after a single axotomy. Nevertheless, the axons regenerated at the accelerated rate typically seen in conditioned nerves.

We conclude that 1) elevated levels of fast axonal transport become essential for sustained outgrowth of goldfish optic axons after a week of regeneration; and 2) the elevated levels of fast transport seen after a single lesion of the goldfish optic nerve are sufficient to support outgrowth at an accelerated rate.

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## GLIAL AND NEURONAL MEMBRANE COMPOSITION II

331.1 STEROID HORMONES STABILIZE LIPOSOMAL MEMBRANE FROM ALTERATION BY TUBULIN. Paul Y. Sze, Ellen C. Armstrong, Grace G. Deanin and Malcolm W. Gordon. Chicago Med. School, North Chicago, IL 60064, Univ. of Conn., Storrs, CT 06268 and Univ. of New Mexico Medical School, Albuquerque, NM 87131.

There is an increasing body of evidence that the plasma membrane is a cellular target site of steroid hormone actions, in addition to genomic mechanisms involving intracellular receptors. In central neurons, specific binding sites for glucocorticoids and gonadal steroids have recently been identified in synaptosomal plasma membrane. To understand steroid interaction with membranes, we have used an artificial membrane system consisting of a single lipid and a single protein. Liposomes (small unilamellar vesicles) were prepared from dipalmitoylphosphatidylcholine (DPPC) with 0.1 M calcein encapsulated. The addition of tubulin to the liposomes resulted in an alteration of bilayer conformation, with subsequent leakage of the encapsulated dye at phase transition. Dye release was a sigmoidal function of tubulin concentration, with maximal release obtained at 0.1 mg/ml. We found that when corticosterone was incorporated into the liposomal membrane, the bilayer became resistant to alteration by tubulin, as determined by dye release. Binding of tubulin was not reduced by steroid incorporation, nor was the phase transition temperature of the bilayer significantly changed. Other hormonal steroids examined (including hydrocortisone, testosterone, 17β-estradiol and progesterone) showed similar stabilizing effects on the bilayer toward tubulin, and their effects were found to be far greater than that of cholesterol, a substance known for its role in regulating membrane fluidity. Most interestingly, among the hormonal steroids examined, the glucocorticoids showed the strongest effects in stabilizing the bilayer against the tubulin-induced alteration. For example, corticosterone as low as 0.002 M% was sufficient to produce an effect comparable to that obtained by cholesterol at 10 M%. The effect of hydrocortisone at 0.008 M% was similar to that from cholesterol at 0.1 M%. On the other hand, deoxycorticosterone, a synthetic steroid that antagonizes many of the biological activities of glucocorticoids, was found to further destabilize the bilayer toward the tubulin-induced effect. It appears from our data that at least in the interaction between DPPC bilayer and tubulin, glucocorticoids act on the bilayer as "super-cholesterols". We propose that in cell membranes, the steroid hormone binding sites (possibly a phospholipoprotein) serve as carriers and determine specificity, whereas the primary actions of the hormones are on lipid bilayers.

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331.2 INCORPORATION OF DOCOSAHEXAENOIC ACID INTO GLYCEROLIPIDS AND CONVERSION TO LIPOXYGENASE REACTION PRODUCTS IN THE RAT BRAIN IN VIVO AND IN MONKEY BRAIN SYNAPTOSOMES IN VITRO. Dale L. Birkle* and Nicolas G. Bazan. (SPON: H.E.P. Bazan). Louisiana State Univ. Med. Ctr., LSU Eye Center, New Orleans, LA 70112.

Docosahexaenoic acid (22:6, n-3) is highly enriched in the glycerolipids of synaptic plasma membranes, and accumulates as free 22:6 following ischemia, electroconvulsive shock and bicuculline-induced status epilepticus. This accumulation may be mediated by the phospholipase $A_2$-induced release of 22:6 from membrane lipids. Recently, we reported the conversion of 22:6 via a lipoxygenase to oxygenated metabolites, including 11-hydroxy-docosahexaenoic acid (11-HDHE), in a central nervous system tissue, the canine retina (Biochem Biophys Res Comm 1984, 125:741). Production of HDHEs is not affected by inhibitors specific for lipoxygenases that use arachidonic acid (20:4,n-6) as a substrate, therefore a distinct mechanism for the production of HDHEs has been proposed. Because of the specific enrichment of 22:6 in synaptic plasma membrane, it is of interest to investigate the metabolism of this highly unsaturated fatty acid in the brain. [1-$^{14}$C]22:6 (1 μCi, $Na^+$ salt) was injected bilaterally into rat lateral cerebral ventricles. At 5, 15, 30, 60 and 120 min after injection, rats were decapitated and the heads frozen in liquid nitrogen. Labeling of phosphatidylcholine, phosphatidylethanolamine and triacylglycerol was most active, and incorporation reached a plateau at 60 min. A second group of rats was injected intraventricularly with [1-$^{14}$C]22:6 and 60 min later treated with 10 mg/kg (i.p.) bicuculline. Rats were killed by high power microwave irradiation (6.5 kW for 1.25 sec) 5 min after drug treatment, during the status epilepticus phase. Lipid extracts were prepared from cerebrum. Extracts were analyzed by high performance liquid chromatography to determine the presence of lipoxygenase reaction products (HDHEs). Approximately 6% of the total radioactivity of the lipid extract was lipoxygenase reaction products. The profile of products was different from the profile observed previously in the canine retina. Preliminary evidence suggests an enhanced production of HDHEs in cerebrum from bicuculline-treated rats. A third experiment explored the synthesis of lipoxygenase reaction products of 22:6 and incorporation into glycerolipids in synaptosomes prepared from monkey cortical grey matter. Synaptosomes were incubated for 60 min at 37°C in the presence of 0.5 μCi [1-$^{14}$C]22:6, and in the presence or absence of 45 mM $K^+$. Characterization of the metabolism of 22:6 in synaptosomes, and the possible significance of 22:6 accumulation and synthesis of HDHEs in neurons will be discussed. Supported by NIH EY07073 and The Esther A. and Joseph Klingenstein Fund, Inc, New York.

331.3 HEME COMPETITIVELY INHIBITS BINDING OF FLUORESCENT BIS-ANS TO MYELIN BASIC PROTEIN. Stephen J. Morris*, Diane Bradley*, Peter E. Braun and Gerald L. Stoner (SPON: R.L. Irwin). Lab. of Experimental Neuropathology, NINCDS, NIH, Bethesda, MD 20205, USA and Dept. of Biochem., McGill Univ., Montreal, Quebec H3G 1Y6, Canada.

Although the traditional view of purified myelin basic protein (MBP) structure in solution is that of a random coil polymer [1], some experimental evidence suggests that MBP has definite long-range order [2,3]. A model of MBP conformation based on analysis of the amino acid sequence predicts the presence of a 5-strand antiparallel β-sheet [4].

Our fluorescence spectral data of the sole Trp residue in MBP shows that it acts as the acceptor for resonance energy transfer (RET) from one or more Tyr residues. Both Trp fluorescence and RET can be collisionally quenched by $I^-$ and acrylamide (A), but not by $Cs^+$ or $Co^{2+}$, implying that the fluorophores are in an exposed, positively charged environment. The quenching coefficients (Q) for $I^-$ and A are ~15 $M^{-1}$. Heme, which has previously been reported to bind to MBP [5,6], also quenches both Trp fluorescence and RET with an apparent Q of $1.0 \times 10^5$ $M^{-1}$, some 6,700-fold larger, suggesting [7] that heme has relatively high affinity for MBP. The negatively charged fluorescent probes 1,8-ANS and 2,6-TNS have been reported to bind to MBP with $K_d$ of $2\text{-}3 \times 10^{-5}$ M and $B_{max}$ of ~1:1 [2,8]. Our data produces similar values. Bis-ANS binds to albumin more avidly and specifically than ANS or TNS [9]. We find an apparent $K_d$ and $B_{max}$ for bis-ANS binding to MBP of $\sim 4 \times 10^{-6}$ and 0.5:1. Heme competitively inhibits bis-ANS binding with an apparent $K_i$ of $\sim 6 \times 10^{-7}$ M.

The affinities of ANS and heme for MBP, along with the RET and fluorescent amino acid quenching data demonstrate that MBP contains considerable structural specificity, implying long-range interactions in the molecule as predicted. Tyr → Trp RET demands a maximum separation of these fluorophores of <15 Å. The binding site for the heme and ANS derivatives must be within the same distance constraints. ANS prefers positively charged, hydrophobic environments. The first requirement is satisfied by the β-sheet model which places Tyr-14 ~5 Å from Trp-117 on an adjacent strand. The second and third requirements would be met if the heme/ANS binding site were located on the β-sheet near these residues.

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331.4 IS THE C-TERMINUS OF JC, BK, AND SV40 VIRUS LARGE T-ANTIGENS ANCESTRALLY RELATED TO MYELIN BASIC PROTEIN Gerald L. Stoner. Lab. of Exp. Neuropathol., NINCDS, NIH, Bethesda, MD 20205.

Myelin basic protein is phosphorylated in a ProArgThrProProPro sequence on Thr-99[1]. A similar sequence occurs in the C-terminus of the large T antigens of JC and BK virus (ProLysThrProProPro) and of SV40 virus (ProProThrProProPro)(699-704)[2]. The latter is known to be phosphorylated on Thr-701[3]. Beyond these sites, there is no obvious homology between MBP and the primate papova virus large T antigens. However, in severely immunocompromised hosts both JC and SV40 virus can infect oligodendroglial cells. In man JC virus causes the demyelinating disease, progressive multifocal leukoencephalopathy(PML)[4]. Therefore, the possibility of a distant ancestral relationship was explored with the SEQDP program[5]. SEQDP compares the optimal alignment of two sequences with the average scores of 100 randomized sequences of the same amino acid composition. Evolutionary distance is measured by the z value ([score - mean score]/standard deviation)[6]. It was found that the central segment of MBP (60 residues) was more closely related to the papova virus T antigen C-terminal sequences than to any of 90 other triproline-containing sequences in the NBRF protein sequence data base. The z values obtained (~2.5-5) are consistent with an ancestral relationship[6]. It is concluded that interaction with an unknown protein kinase may have conserved these sites in sequences which have otherwise diverged widely. Similar analysis of several sites of O-glycosylated threonine residues which also precede triproline sequences indicated no detectable ancestral relationships, suggesting that those similarities represent convergence rather than divergence. It seems most probable that triproline sequences have been utilized as recognition sites for post-translational modification of nearby residues because they are inherently rigid. This rigidity may promote specific recognition of an otherwise flexible portion of the molecule[7]. Regardless of the evolutionary mechanisms which may have been involved in their selection, the sharing of sites between papova virus T antigens and MBP suggests two novel mechanisms of demyelination: (1) During oligodendrocyte infection the T-antigen site might compete for the unknown protein kinase which phosphorylates Thr-99 of MBP. (2) If the T-antigen triproline sequence is immunogenic, it might induce an immune response cross-reactive with MBP and capable of triggering CNS pathology in the absence of oligodendrocyte infection.

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331.5 EXPRESSION OF MYELIN PROTEINS IN DEVELOPING RAT SCHWANN CELLS A. F. Hahn*, B. Kachar*, H. deF. Webster. Lab. of Experimental Neuropathology, NINCDS, NIH, Bethesda, MD 20205.

Previous immunocytochemical studies of $P_2$ protein describe a selective distribution of immunoreactivity in myelin sheaths surrounding large axons of rat peripheral nerves (Trapp et al., 1979; Winter et al., 1982). To better characterize Schwann cell expression of $P_2$ protein during development, we examined the appearance and density of anti-$P_2$ immunoreactivity and related them to axon and myelin sheath size in a well defined fiber population. Myelin protein expression was studied immunocytochemically in rat cranial nerves during development (postnatal day 1,2,3,4,5,8,15,20). At the level of the sphenoid bone, cranial nerves III, IV, and VI lie within the dura surrounding V and functionally defined fiber populations can be studied in single transverse semithin or thin sections. We found this site well suited for studying myelin protein immunoreactivities and their relationships to: (1) onset of myelination (2) axon diameter (3) myelin spiral length (4) fiber function. Polyclonal antisera reacting specifically with $P_0$ glycoprotein and basic proteins $P_1$ and $P_2$ were used in the PAP method to immunostain serial semithin epon sections. Immunoreactivity of individual sheaths in the VI nerve was measured by densitometry. Numbers of compact myelin lamellae, myelin spiral lengths, and axon diameters were determined on electron micrographs of adjacent thin sections. At birth, anti-$P_0$ staining was found on sheaths with 2 or more compact lamellae; neither $P_1$ nor $P_2$ immunoreactivity was observed. On day 2, myelin sheaths with 4-5 and 5-8 lamellae were stained respectively by anti-$P_1$ and anti-$P_2$. On day 3, the percentages of myelinated fibers that were immunostained were substantially higher, $P_0 > P_1 > P_2$. By day 4, anti-$P_0$ and anti-$P_1$ immunoreactivity was present in 95% of myelin sheaths; 35% were stained by anti-$P_2$. For $P_2$, staining intensity and the percentage of myelin sheaths stained continued to increase; by day 20, 85% were anti-$P_2$ positive. Staining intensities were not uniform in all sheaths in identically processed sections. Intensity variation was greatest with anti-$P_2$, less with anti-$P_1$, and least with anti-$P_0$; staining became more uniform with increasing age. Preliminary quantitative results suggest that intensity of anti-$P_2$ immunoreactivity correlates better with the amount of myelin present (myelin spiral length and number of myelin lamellae) than with axon diameter. Differences in detection of immunoreactivity and its intensity may reflect relative amounts of this minor PNS myelin constituent that can be detected by the method rather than selective expression of $P_2$ by certain myelin-forming Schwann cells. Qualitative comparisons of VI and V indicate that expression of $P_2$, $P_1$, and $P_0$ myelin proteins is independent of fiber function.

We thank J.N. Whitaker for $P_1$-and $P_2$-antisera, B.D. Trapp for $P_0$-antiserum and J. Fex for the use of the digital image processor.

331.6 SOME NEW BASIC PROTEINS IN CHICKEN POSTSYNAPTIC DENSITIES. Z.Y. Yang* and J.A. Babitch. Dept. of Chemistry, Texas Christian University, Fort Worth, Texas 76129.

Postsynaptic densities (PSD) were previously reported to contain 20 to 30 proteins, based on one-dimensional SDS-PAGE. So far only a few of them have been identified. Most of their physical and biochemical properties are still to be investigated. PSDs were obtained from 10-day-old chicken brains by treatment of crude synaptosomes with 0.5% Triton X-100 and then were further purified on sucrose gradients. The isoelectric focusing gel system, improved by substitution of 3[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate(CHAPS) for NP-40 and increased cathode buffer concentration, produces better resolution and a stable pH gradient spanning the range 4-9. Some new basic proteins were detected in these improved IEF-SDS two-dimensional gels. They are: 82K (pI>9.1); 58K (pI 8.1 to 8.8 heterogeneous); 40K (pI 9.0) and 24K (pI 8.9). Nonequilibrium pH gradient-SDS gels further show some extremely basic proteins with pIs higher than 9: 81K, 53K, 47K, 45K, 36K, and two at 34K. All together we observed 11 major basic proteins. They amount to about half of the total PSD proteins appearing on these gels. These basic proteins are distinguishable from those of brain mitochondria, the major potential contaminants. This is further confirmed by experiments mixing $^3H$-labeled liver mitochondrial proteins with synaptosomes during PSD isolation. Some PSD proteins can be phosphorylated by endogenous protein kinase(s) in the presence of $Ca^{2+}$ and [$\gamma$-$^{32}$P]ATP. A major basic protein of mol. wt. 82K is strongly phosphorylated along with some other basic proteins. However, none of the others are major proteins. To evaluate their $Ca^{2+}$- binding properties, PSD proteins on nonequilibrium pH gradient-SDS gels were electrophoretically transferred to nitrocellulose membranes, which were then incubated with $^{45}Ca^{2+}$ for 30 min. Excess $^{45}Ca^{2+}$ was washed off with double distilled water. The autoradiograms show $Ca^{2+}$-binding proteins of 70K (probably a subunit of neurofilaments or a microtubule associated protein), 61K ($\alpha$-tubulin), 54K ($\beta$-tubulin), 46K (actin) and an acidic protein with low molecular weight, probably calmodulin. No basic proteins showed $Ca^{2+}$-binding capacity. Supported by the TCU Research Fund and NIH.

331.7 MOUSE NEUROBLASTOMA CELLS: A MODEL SYSTEM FOR STUDYING SPECTRIN EXPRESSION S.R. Goodman*, I.S. Zagon, D.B. Coleman*, and P.J. McLaughlin (SPON: T. Lloyd). Dept. of Physiology and Anatomy, The M.S. Hershey Medical Center of The Pennsylvania State University, Departments of Physiology and Anatomy, Hershey, PA 17033

To begin the study of the synthesis and assembly of the spectrin based cytoskeleton in a neuron-like cell type, we have chosen S20Y mouse neuroblastoma cells as a model system. To study expression of neuroblastoma spectrin, 20 million log phase (8-10% differentiated) or confluent phase (55-60% differentiated) cells were incubated in Eagles EMM-unlabelled methionine + $S^{35}$ methionine (200 μCi/ml, 1126 Ci/mmole) for 4h at 37°C. Cells were solubilized in triton X-100 at high ionic strength as described elsewhere (Glenney, J.R. and Glenney, P., Cell 34: 503, 1983). Addition of antibodies against mouse brain spectrin under these stringent conditions allowed specific immunoprecipitation of $S^{35}$-labelled neuroblastoma spectrin with a 240 kDal α subunit and a 235 kDal β subunit in a 1:1 molar ratio. No $S^{35}$-labelled neuroblastoma spectrin (rbc type) could be immunoprecipitated with anti-mouse rbc spectrin IgG. Chymotryptic two-dimensional peptide mapping analysis of $S^{35}$-labelled or $I^{125}$-labelled neuroblastoma spectrin demonstrated that the 240 kDal and 235 kDal subunits are distinct from each other, share little overlap with mouse rbc spectrin α and β subunits, but are nearly identical to mouse brain spectrin α and β subunits respectively. Indirect immunofluorescent staining of neuroblastoma cells with an antibody against mouse brain spectrin demonstrated a brightly stained cell body, no nuclear staining, and staining of all processes with bright staining of the growth cones. Intense perinuclear staining of fibers which radiated into the neurites was observed in most cells. The model system described should allow us to study the synthesis and assembly of spectrin subunits and associated proteins in a homogenous neuron-like cell system. Supported by NIH grants NS-19357 and HL-26059 to SRG, and NS-21246 to ISZ. SRG is an Established Investigator of the American Heart Association.

331.8 PHOSPHORUS METABOLISM AND $^{3}H$-SAXITONIN BINDING ASSOCIATED WITH PHOSPHOLIPASE $A_2$-INDUCED CONDUCTION BLOCK. J.W. Kaskow, L.G. Abood, W. Hoss and R.M. Herndon. Center for Brain Research, University of Rochester School of Medicine, Rochester, NY 14642.

Recently, an investigation of enzymatic treatments on nerve conduction revealed that phospholipase $A_2$ ($PLA_2$), which disrupts myelin, causes profound conduction block; β-glucosidase and proteases were without effect (Kaskow et al., Soc. Neurosci. Abstr. 10:1179, 1984). The objective of this study was to investigate the biochemical changes responsible for the conduction block associated with exposure of desheathed bullfrog sciatic nerves to $PLA_2$ from bee venom. At a concentration of $PLA_2$, which produced at least a 50% block in nerve conduction, there resulted a 45% decrease in both phosphatidylcholine (PC) and phosphatidylethanolamine (PE) with no significant change in phosphatidylserine. In addition, there occurred a 58% and 78% decrease in ATP and phosphocreatine (PCr), respectively, and about 50% increase in AMP. Using $^{32}P$-orthophosphate, it was demonstrated that $PLA_2$ decreased $^{32}P$ influx, increased $^{32}P$ outflux, while having no effect on ATP and PCr turnover. $^{3}H$-Saxitonin binding was inhibited 85% by $PLA_2$ treatment. These findings suggest that $PLA_2$, by hydrolyzing PC and PE, disrupts sodium channels and depletes high energy phosphates, leading to conduction failure because of a decrement in sodium conductance and an inability to maintain ionic gradients. Supported by HHS Grant DA 00464 (LGA).

331.9 A RARE CELL SURFACE ANTIGEN FOUND ON MOTOR NEURONS P.D. Kushner, H. Sternberg*, D. Stephenson*, and G. Cole* ALS and Neuromuscular Research Foundation, Pacific Medical Center, San Francisco, CA. 94115.

We have identified a cell surface component of motor neurons which is shared with few other CNS neurons and is present in phylogenetically diverse species. We discovered this component using an antibody made to Torpedo electroplax synaptosomes. Electron microscopic immunolocalization reveals that this component resides on the plasma membrane of neurons and appears externally distributed. This external distribution has been confirmed on a differentiated teratoma culture of human origin, NT2/D1. Molecular weight analysis in the presence of reducing agent, reveals that the epitope is found on two polypeptides of electrophoretically separated, Torpedo synaptosomes, $M_r$ = 67,000d and 180,000d. Without reduction two different molecular weight species are observed, 130,000d and over 250,000d. These data, along with the observation that antibody recognition is destroyed upon pretreatment of Torpedo synaptosomes with trypsin, is indicative that the epitope resides on proteinaceous molecules. The determinant appears to be partially concealed by disulfide linkages because, upon thiol-reduction, the antibody signal is enhanced (both by radioimmunoassay and immunogel analyses). Because the molecular weight findings are similar to published accounts of the Torpedo acetylcholinesterase catalytic subunit (67,000d, reduced and 130,000d, unreduced) and because esterase is abundant in Torpedo synaptosomes, we tested the possibility that this antibody might recognize a component with esterase catalytic activity. We performed immunoprecipitation and subsequent enzyme activity analyses. We find that, indeed, this antibody does precipitate acetylcholinesterase activity from Torpedo synaptosomes and that this activity is inhibited by BW284C51 and does not utilize butyrylthiocholine as substrate.

This work was supported by ALS Society of America and National ALS Foundation.

331.10 CHARACTERIZATION OF A POLYSPECIFIC ANTISERUM THAT DISRUPTS NEURONAL-ASTROGLIAL INTERACTIONS IN VITRO. J.C. Edmondson, J.M. Alter* and M.E. Hatten. Department of Pharmacology, New York University Medical Center, New York, NY 10016.

Our laboratory has developed a polyspecific antiserum that quantitatively disrupts specific neuronal-astroglial interactions in monolayer cultures from early postnatal mouse cerebellum, as reported previously (Society for Neuroscience Abstracts, 10:759, 1984). Fab fragments prepared from the antiserum randomize the distribution of neurons with respect to glial cells in culture and also cause stunting of glial process outgrowth. Here we report further cytological and biochemical characterizations of this immune activity.

By immunofluorescence localization, the antiserum stains the entire surfaces of neurons and astroglia in cerebellar cultures, purified cerebellar neurons, purified cerebellar astroglia, PC12 cells and some glioma lines, but not PTK2 (kangaroo kidney epithelial) cells. By HRP labelling at the electron microscopic level, the antiserum stains the surfaces of both neurons and astroglia (identified as containing 10 nm filaments), and also heavily labels the interfaces between the two cell types.

Preabsorption of the antiserum with whole dissociated cerebellar cells from normal cerebellum results in loss of the biological activity. Preabsorption with PC12 cells, a cell type that does not interact specifically with cerebellar cells in vitro, does not remove the biological activity. Preabsorption with cells from the midline portion of the homozygous weaver mutant mouse, a mouse which suffers defects in astroglial differentiation and glial-guided migration, also does not remove the biological activity.

When cultures are grown in the presence of $^{3}H$-fucose, solubilized gently in Triton X-100, immunoreacted with the antiserum, precipitated with Protein A Sepharose and run on SDS-PAGE, at least five prominent species are seen in autoradiograms, indicating that the antiserum recognizes a family of glycoproteins. The three higher molecular weight components, 250, 210 and 140 kd, are removed if the antiserum is preabsorbed with PC12 cells. The remaining bands are 105 kd and a diffuse band centered around 85 kd. Immunoprecipitation with weaver-preabsorbed antiserum is currently in progress. Supported by NIH grant NS15429 (MEH).

331.11 STRUCTURES OF TWO NEURONAL CELL ADHESION MOLECULES B.C. Sorkin*, M. Grumet*, B.A. Cunningham* and G.M. Edelman. Dept. of Developmental and Molecular Biology, The Rockefeller University, NYC, NY 10021

The neural cell adhesion molecule, N-CAM, mediates neuron-neuron and neuron-myotube adhesion. We have refined our topographical model of N-CAM, consistent with a variety of biochemical and molecular biological data. The most distinguishing feature of N-CAM is the presence of polysialic acid on one or more of the three asparagine-linked oligosaccharides in the central domain of the molecule; sugars other than sialic acid were sulfated in some of these carbohydrates, both in vitro and in ovo. In contrast, primary cultures of neurons incorporated sulfate into N-CAM, but not into that material released by endoglycosidase F. The carboxy terminal third of the molecule that is associated with the cell membrane contains covalently bound fatty acid, phosphoserine and phosphothreonine in both polypeptide chains. The two components yield different phosphopeptides after trypsinization, and the ratio of phosphoserine to phosphothreonine in the larger species is almost four times as high as in the smaller. These and other data indicate that the amino acid sequences of the two polypeptides differ in this part of the molecule.

The neuron-glia cell adhesion molecule, Ng-CAM, which is present on the surface of post-mitotic neurons, mediates neuron-glia adhesion and may play a role in neuron-neuron adhesion. Ng-CAM contains less sialic acid than N-CAM and consists of three polypeptide chains with Mr's of 200,000, 135,000 and 80,000. The components of Mr=135,000 and 80,000 are immunologically related to the Mr=200,000 species but not to each other and only the Mr=200,000 and 80,000 components are phosphorylated in vitro, suggesting that the Mr=135,000 and 80,000 components are derived from the Mr=200,000 polypeptide. Despite their differences, N-CAM and Ng-CAM share at least one oligosaccharide determinant that is also present on myelin-associated glycoprotein. Methods similar to those used with N-CAM have been used to generate a topographical model of Ng-CAM.

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331.12 MODULATION OF NEURAL CELL ADHESION MOLECULE EXPRESSION DURING IN VITRO DEVELOPMENT AND MALIGNANT TRANSFORMATION. D.R. Friedlander, R. Brackenbury* and G.M. Edelman. Dept. of Molecular Biology, Rockefeller University, 1230 York Ave., New York, N.Y. 10021.

During normal development, the neural cell adhesion molecule N-CAM changes in amount and structure. In the latter, a sialic acid-rich embryonic, or E form, changes to several adult, or A forms, that have less sialic acid (E to A conversion). The potential functional significance of these examples of cell surface modulation (Edelman, G.M., Science, 192:218-226, 1977; PNAS 81:1460-1464, 1984) is suggested by experiments in which the binding rate of N-CAM reconstituted into synthetic vesicles is strongly dependent on the amounts and chemical form of the molecule (Hoffman, S. & G.M. Edelman. PNAS 80:5762-5766, 1983). To gain insight into the control of N-CAM expression, we have examined two in vitro systems which exhibit prevalence modulation and E to A conversion.

Changes in the amount of N-CAM were observed in neuroepithelial cells from 7 day chick embryos after transformation by Rous sarcoma virus (RSV). The transformed cells were morphologically altered, were more motile, and, as revealed by specific immunoblotting, immunofluorescence, and immunoprecipitation analyses, expressed 7-10 fold less N-CAM than uninfected cells or cells infected with transformation-defective RSV. Expression of N-CAM was temperature-dependent in cells infected with mutants of RSV that are temperature-sensitive for transformation. Experiments using N-CAM cDNA probes suggest that the alteration in amount of N-CAM occurs at the transcriptional level.

E to A conversion was observed in explants of mouse cerebellum, chick spinal cord and chick retina. After 3-6 days in culture, increased proportions of A forms were synthesized, and the rate of E to A conversion and the proportions of the different A forms were similar to those observed in vivo. Pulse-chase experiments, metabolic labeling with $^{3}$H-glucosamine and treatment with neuraminidase indicated that the synthesis and glycosylation of the N-CAM polypeptide chains in vitro were normal. In long term pulse-chase experiments, the E form of N-CAM that was synthesized during the first day after explantation persisted for several days, although in reduced amounts. At these later times, newly synthesized N-CAM was predominantly in A forms, indicating that in cultured neural tissue, the E form of N-CAM is not processed into A forms but is gradually degraded and replaced by newly synthesized A forms. Together, these results indicate that N-CAM expression may be altered by changes in mRNA transcription or stability, and post-translationally, by changes in levels or activity of sialyltransferases. These in vitro systems should be useful in defining the molecular mechanisms involved in the control of N-CAM expression.
This work was supported by U.S. Public Health Service grants HD-09635, HD-16550, AI-11378.

## BIOLOGICAL RHYTHMS IV

332.1 INTRACELLULAR CURRENT INJECTION INTO PUTATIVE PACEMAKER NEURONS PHASE SHIFTS THE BULLA OCULAR CIRCADIAN PACEMAKER. D.G. McMahon and G.D. Block, Dept. of Biol., University of Virginia, Charlottesville, Va. 22901

In an effort to elucidate the mechanisms of entrainment of circadian rhythmicity we have investigated the role of changes in transmembrane potential in phase shifting the ocular circadian pacemaker of the marine mollusc Bulla gouldiana. The Bulla eye contains a circadian pacemaker which expresses a circadian rhythm in the frequency of spontaneous optic nerve impulses in vitro. A group of electrically coupled basal retinal neurons (BRNs) have been identified as the likely site of the circadian oscillator within the retina. Intracellular recordings from these neurons reveal that they respond to entrainment stimuli with changes in membrane potential (e.g. depolarize to light). It has been proposed by Eskin (1972) and ourselves (1984) that changes in membrane potential could play a role in phase shifting molluscan circadian pacemakers. We now report that changing BRN membrane potential by intracellular current injection directly into the putative pacemaker neurons is sufficient to phase shift the ocular circadian pacemaker. In addition, suppressing light-induced depolarizations by injection of hyperpolarizing current substantially reduces light-induced phase shifts.

Pairs of Bulla eyes were dissected from the animal in the late subjective day. Eyes were maintained in sterile ASW and darkness at 15 °C. At various times following dissection a BRN in one eye was penetrated with a glass capillary microelectrode filled with 4M KAc (80-120 MΩ). Following impalement, depolarizing or hyperpolarizing current was injected into the BRNs. In some experiments both eyes were exposed to light (5000 lux) while hyperpolarizing current was injected into the BRNs of one eye to suppress the light-induced depolarization. Phase shifts were measured on the second circadian cycle following treatment.

Injection of depolarizing current mimics the phase shifting action of light, producing phase delays in the early subjective night (SN, mean phase shift=-1.6h), advances in the late SN (+1.5h), and no shift during the subjective day (SD,+0.1h). Injection of hyperpolarizing current produces advances in the late SD (+1.2h), and no shift in the early SN (0.0h). Injection of hyperpolarizing current during light pulses in the early SN reduced the light induced phase delay by 0.9h.

Either depolarizing or hyperpolarizing the BRNs is sufficient to phase shift the pacemaker. Interestingly, these treatments produce advances approximately 12h out of phase, suggesting that they are acting on different phase response curves. The results suggest that changes in BRN membrane potential play a critical role in the entrainment of the Bulla eye. NS-15264 to G.D.B.

332.2 CIRCADIAN PACEMAKER COUPLING IN BULLA: EFFERENT OPTIC NERVE IMPULSES INDUCE PHASE SHIFTS IN OCULAR PACEMAKER. G.D. Block, L.-H. Yen*, A.E. Lusakas*. Dept of Biology, University of Virginia, Charlottesville, VA. 22901.

The eyes of the molluac, Bulla, contain circadian pacemakers which are mutually coupled (Science 221:87). An analysis of the timing of interocular coupling signals indicates that phase shifts occur during the time that the contralateral eye spontaneously produces compound action potentials (CAPs- Neurosci. Abstr. 10:89.2). These impulses are conducted through the cerebral ganglion and generate epsps in basal retinal neurons- putative circadian pacemaker cells (Neurosci. Abstr. 10:89.3). The coincidence of ocular phase shifts and spontaneous impulse activity suggests that CAPs are the coupling signal. To test this hypothesis we attempted to phase shift the ocular rhythm with efferent optic nerve impulses generated either by electrically stimulating the optic nerve or by illuminating the contralateral eye.

For electrical stimulation eyes were removed from 12 animals and the optic nerve of one eye from each pair stimulated (1/10 Hz) for 2 hr beginning at CT 12 or CT 20. Intracellular recording experiments indicated that electrically evoked impulses generated epsps in basal retinal neurons. Optic nerve activity of stimulated and control eyes was then recorded in darkness for 48 hr and the phase difference between the two CAP rhythms measured on the second cycle. We found that stimulation at CT 12 generated a -0.8 hr mean phase delay (N=6) compared with the unstimulated control eye while stimulation at CT 20 induced a mean +0.8 hr advance (N=6).

Efferent impulses were also generated by illuminating the contralateral eye. Each eye and attached ganglia were placed in separate compartments of a "Y" dish in which slits were cut for the optic nerves. One compartment was illuminated with a miniature light guide while the seawater in the other eye compartment was made opaque with nigrosin dye. Control preparations consisting of detached eyes were placed in identical chambers. Two hr light pulses were delivered at CT 12. At the end of the light pulse both eyes were isolated and CAP activity recorded in darkness for 48 hr. We found a mean phase delay of -0.9hr (N=4) between pulsed and unpulsed detached eyes while the mean difference between attached eyes was only -0.1 hr (N=4).

These results suggest that efferent impulses phase shift the ocular pacemaker. While entrainment mechanisms are poorly understood, it appears that depolarization of basal retinal neurons is a critical step in the light entrainment pathway (McMahon & Block, this volume). Since spontaneous and induced efferent signals likewise depolarize basal retinal neurons, transmembrane potential may be similarly important in the coupling pathway. Supported by NS15264 and RCDA NS00714.

332.3 FMRFAMIDE BLOCKADE OF OPTIC NERVE PACEMAKER ACTIVITY AND FMRFAMIDE IMMUNOREACTIVITY OF OPTIC NERVE EFFERENT FIBERS AND RETINAL NEUROPIL OF BULLA AND APLYSIA. Jon W. Jacklet. Neurobiology Research Center and Dept. Biology, SUNYA, Albany, NY 12222.

The amidated tetrapeptide FMRFamide isolated from molluscan ganglia functions to regulate the cardiovasular system and modulate activity of specific neurons. FMRFamide-like immunoreactivity of neurons is reported for animals from coelenterates to mammals, and a gene that encodes FMRFamide is identified in Aplysia neurons (Schaefer et al., Cell in press, 1985).

The isolated eyes of Bulla and Aplysia contain circadian pacemaker neurons, whose synchronized endogenous activity is recorded as compound action potentials (CAPs) from the optic nerve (ON) of isolated eyes. FMRFamide in Hepes buffered (pH 7.8) artificial sea water superfused over the eye reversibly blocks the CAPs. In Bulla (n=6) CAP frequency is reduced by 0.2 at 10 nM FMRFamide, by 0.5 at 100 nM and completely blocked at 1 μM. In Aplysia (n=8) CAP frequency is reduced by 0.3 at 1 μM and by 0.7 at 10 μM.

Immunocytochemistry using FMRFamide antisera obtained from Immuno Nuclear Corp. (antibody 72432) and rhodamine-labeled secondary antisera in a procedure similar to one used by Kistler et al. (J. Neuroscience 5:72-80, 1985), on sectioned Bulla neural tissue resulted in labeling of specific neurons in the cerebral and pedal ganglia. Fibers in the neuropil, interganglionic commissures and peripheral nerves were also labeled. About 10 efferent fibers (1 μm dia) in the ON were labeled. They extend to the retinal neuropil where they form a network of varicose endings. Some of them branch in the nerve and send terminal processes into the nerve sheath as well. Labeling was especially high in fibers within the neuropil adjacent to the circadian pacemaker neurons. In addition to the fibers, there are 2-3 small (< 10 μm) immunoreactive retinal neurons near the boundary between the photoreceptors and the pacemaker neurons.

In contrast only a few immunoreactive fibers were observed in the Aplysia retina, although there were immunoreactive neurons and fibers in the central ganglia. This correlates with the relative insensitivity of the Aplysia eye to FMRFamide. The high sensitivity of Bulla CAP activity to superfused FMRFamide and the intense FMRFamide-like immunoreactivity of the small intrinsic neurons and efferent fiber terminals in the neuropil adjacent to the circadian pacemaker neurons suggests a physiological role for FMRFamide release in control of the circadian pacemaker of Bulla.

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332.4 VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) AND PEPTIDE HISTIDINE ISOLEUCINE (PHI) CO-LOCALIZE IN NEURONS OF THE RAT SUPRACHIASMATIC NUCLEUS (SCN). R.Y. Moore, J. Speh and J.P. Card. Dept. of Neurology and Neurobiology, SUNY-Stony Brook, NY 11794.

VIP is a 28 amino acid polypeptide which is contained within neurons of a number of functionally distinct systems in the central nervous system. We have demonstrated previously that the ventrolateral subdivision of the SCN contains a large number of VIP-immunoreactive neurons and receives dense and overlapping visual inputs from the retina and lateral geniculate complex (cf. Moore, '83 for review). Recently, Tatemoto and Mutt ('81) isolated and characterized a glucagon-secretin-like peptide, PHI. This 27 amino acid peptide exhibits extensive sequence homology with VIP and its distribution in rat brain is very similar to that of VIP (Beinfeld, '84). PHI-like peptides and VIP also appear to be produced from a common precursor (Itoh et al, '83) indicating that both peptides should be found in the same neurons.

The present study was undertaken to test this hypothesis and to further characterize the distribution of VIP neurons and their efferent projections in the rat hypothalamus. Antibodies to VIP and PHI were obtained commercially and the distribution of each peptide was analyzed immunohistochemically in serial coronal sections through the hypothalamus. The distribution of VIP- and PHI-like immunoreactivity is identical and analysis of serial adjacent sections indicates that both antisera are identifying the same neurons. Neurons exhibiting both VIP and PHI immunoreactivity are found in the medial preoptic area and the SCN. The distribution of immunoreactive neurons in the SCN is consistent with our previous observations on the localization of VIP in this nucleus (Card and Moore, '81). Scattered immunoreactive perikarya are present at the rostral and caudal poles of the SCN, but the majority of cells are located in the ventrolateral aspect of the nucleus at intermediate levels of the rostrocaudal axis. The densest concentration of immunoreactive fibers is found in the immediate vicinity of their cells of origin. However, immunoreactive axons also extend throughout the nucleus and into the immediately adjacent hypothalamus. A small group of fibers pass dorsally into the periventricular nucleus and course rostrally towards the organum vasculosum of the lamina terminalis. A second, larger group of immunoreactive fibers pass dorsally through the anterior hypothalamic area adjacent to the periventricular nucleus and terminate along the ventral border of the paraventricular hypothalamic nucleus. One component of this bundle continues caudally through the dorsal aspect of the periventricular nucleus and terminates at the level of the premammilary nuclei. These data demonstrate that VIP and PHI are co-localized in the hypothalamus and provide further information on the efferent projections of the SCN. Supported by NIH grants NS-16304 and NS-19714.

332.5 IMMUNOCYTOCHEMICAL LOCALIZATION OF NEUROPEPTIDE Y IN THE RAT SUPRACHIASMATIC NUCLEUS. J.P. Card and R.Y. Moore. Departments of Neurology and Neurobiology and Behavior, SUNY @ Stony Brook, Stony Brook, New York, 11794.

The suprachiasmatic nucleus (SCN) of the hypothalamus is the primary site in the central nervous system responsible for the generation and regulation of circadian rhythms. Several studies have shown that the SCN receives a projection from the lateral geniculate complex which exhibits neuropeptide Y (NPY)-like immunoreactivity. This projection arises from neurons in the intergeniculate leaflet and terminates selectively within the ventrolateral subdivision of the SCN. Microinjection of NPY in the area of the hamster SCN produces phase shifts in locomotor activity and the direction of the shift is dependent upon the time during the circadian cycle that NPY is administered (Albers & Ferris, 1984). These findings, together with the fact that the geniculate projection is virtually coextensive with the terminal field of retinal afferents, suggest a complex interaction of visual afferents in entraining the neuronal activity of SCN neurons to photic influences of the environment. In the present investigation we have utilized electron microscopic immunocytochemical localization of NPY to analyze the morphology and synaptology of the geniculohypothalamic projection upon the SCN. Consistent with previous light microscopic findings (Card and Moore, 1982; Moore et al., 1984), NPY was localized in varicose axons which are confined to the ventrolateral subdivision of the SCN. Ultrastructural analysis indicates that NPY is present in axon terminals which contain dense mitochondria and a mixed population of lucent, pleomorphic vesicles and small dense core vesicles. Peroxidase reaction product is present throughout labeled terminals but is most intense in the population of dense core vesicles. The dense core vesicles are concentrated at the peripheral border of the axon terminal and have never been observed in the vicinity of synaptic contacts. Immunoreactive terminals establish asymmetric synaptic contacts with distal dendrites and spines of SCN neurons. Other, unlabeled terminals synapse upon these same dendrites and although the source of these terminals has not been established, some of them exhibit distinctive morphology which has previously been associated with retinal afferents in the SCN (Güldner, 1978). These data demonstrate that NPY is contained within a morphologically distinct class of SCN afferents and that the terminals exert their effect on SCN neuronal activity via axo-dendritic synaptic contacts. This is similar to the synaptic organization of retinal afferents in the SCN and provides further information on the means through which visual input to the SCN is integrated in the control of circadian rhythmic phenomena. Supported by NIH grants NS-16304 and NS-19714.

332.6 **ANISOMYCIN, INJECTED EITHER PERIPHERALLY OR DIRECTLY INTO THE SUPRACHIASMATIC NUCLEUS (SCN), INDUCES PHASE SHIFTS IN THE CIRCADIAN RHYTHM OF LOCOMOTOR ACTIVITY OF HAMSTERS.** **S.T. Inouye, J.S. Takahashi and F.W. Turek. Dept. Neurobiology & Physiology, Northwestern University, Evanston, IL 60201.**

**In order to investigate the role of protein synthesis in the generation of circadian rhythms in mammals, we examined the effects of anisomycin, a potent protein synthesis inhibitor, on the circadian locomotor activity rhythm of hamsters. In one study, male hamsters maintained in LD 14:10 were transferred to constant darkness and two weeks later the animals were injected subcutaneously with 12 mg anisomycin in complete darkness at various circadian times. Peripheral injections of anisomycin induced phase-dependent phase shifts in the free-running rhythm of locomotor activity, while saline injections had no effect. Delays were produced when injections occurred between CT22 to CT4 (CT12=onset of activity) with a peak at CT0, while advances were produced following injections around CT9 to CT10. The phase response curve for anisomycin is different from that for light suggesting that these two agents produce phase shifts through different mechanisms. Cycloheximide (10 mg), another protein synthesis inhibitor, yielded a phase response curve similar to that for anisomycin. Alpha-methyl-p-tyrosine methyl ester (150 mg/kg, i.p.) and physostigmine (0.5mg/kg, i.p.) injections, which mimic two known side effects of anisomycin, had no effect on the locomotor activity rhythm. These results suggest that the effects of anisomycin are due to its inhibition of protein synthesis and not to inhibition of either catecholamine synthesis or acetylcholinesterase.**

**Because a likely site of action of anisomycin on the circadian system is the SCN region of the hypothalamus, we examined the effects of microinjecting anisomycin directly into the SCN on the activity rhythm. A microinjection guide assembly consisting of an outer guide tube (23G) and an inner stainless steel pin extending 1.0mm beyond the outer guide cannula was aimed at the SCN and implanted chronically using stereotaxic procedures. Animals were blinded by occular enucleation at the same time. For injections, an inner cannula (30G) connected to a microdrive with polyethylene tubing was placed in the guide cannula. After a stable free running rhythm of locomotor activity was established, anisomycin dissolved in saline (1ul) was injected into the SCN area at around CT0 (the phase at which peripheral injections produce the largest phase delay). Administration of 25ug anisomycin at CT0 induced phase delays if the tip of the cannula was located within 1mm of the SCN. Injections of 5 ug anisomycin induced phase delays if the site of injection was within approximately 0.5mm of the SCN. Taken together these results demonstrate that an inhibitor of protein synthesis perturbs a mammalian circadian system and that the site of action appears to be located in the suprachiasmatic region of the hypothalamus.**

332.7 ENTRAINMENT OF RAT CIRCADIAN RHYTHMS BY DAILY INJECTIONS OF MELATONIN: SYNCHRONIZATION IN CONSTANT LIGHT AND DEPENDENCE UPON THE SUPRACHIASMATIC NUCLEI. Vincent M. Cassone, Michael Chesworth* and Stuart M. Armstrong*. Department of Psychology, La Trobe University, Bundoora, Victoria, Australia 3083.

Although pinealectomy has little effect upon circadian rhythmicity among rodents, daily injections of the pineal hormone melatonin entrain free-running rats in constant darkness (Redman et al., 1983). This study investigated whether rats whose circadian rhythms were disrupted by (1) constant light (LL) or (2) lesions to the hypothalamic suprachiasmatic nuclei (SCN) could be synchronized by daily injections of melatonin. In the first set of experiments, rats were subjected to a paradigm of decreasing scotoperiod until they were unable to entrain, thereby free-running, becoming disrupted or arrhythmic in LL. After 3 weeks in LL, animals were injected every day with either 1 mg/kg melatonin (N=20) or saline-alcohol vehicle (N=10). While no effect of vehicle injections could be determined, melatonin injections entrained free-running rats and synchronized rhythmicity in those rats which became arrhythmic. In the second set of experiments, rats were entrained to an LD 12:12 cycle for 14 days before they either received bilateral lesions to the SCN (N=13) or sham-lesions to the area (N=4). All animals were allowed to recover for 2 weeks in LD before being placed in constant darkness (DD) for another 2 weeks. At 2 weeks, 1/2 of the rats received daily injections of 1 mg/kg melatonin and 1/2 received saline-alcohol vehicle for 60 days. Twelve of 13 lesioned rats became arrhythmic in DD, while no sham-lesioned rats showed long-term effects. Injections of melatonin entrained sham-lesioned rats and the 1 lesioned rat which received an incomplete lesion. Neither saline-alcohol vehicle nor melatonin injections had any effect on SCN-lesioned rats.

These data indicate that high doses of melatonin can synchronize components of activity in rats whose circadian rhythmicity had been disrupted by LL. These synchronizing and entraining effects of melatonin, moreover, appear to depend upon circadian oscillators within the SCN, suggesting that the SCN may be a site of melatonin's action in this experimental situation. Whether this reflects a physiological role for melatonin in circadian organization is as yet to be determined.

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332.8 PHASE-SHIFTING EFFECT OF CARBACHOL ON THE CIRCADIAN ACTIVITY RHYTHM IN THE GOLDEN HAMSTER: DOSE-RESPONSE RELATIONSHIPS. Keith D. Anderson and Fred W. Turek. Dept. Neurobiology and Physiology, Northwestern Univ., Evanston, IL 60201-3816.

Injection of the acetylcholine (ACh) agonist, carbachol, into the lateral ventricle of hamsters exposed to constant darkness (DD) mimics the phase-shifting effect of a light pulse on the freerunning circadian activity rhythm. The phase-response curve obtained for carbachol is similar to that for light pulses, and a daily carbachol injection can entrain the activity rhythm in a manner similar to a daily light pulse. As a basis for further studies on the role of ACh in mediating the effect of light on the circadian system, we investigated the relationship between the dose of carbachol injected and the magnitude of the resulting phase shift under different experimental conditions. A stainless steel cannula was stereotaxically implanted into the lateral ventricle of 3 groups of adult male hamsters exposed to 14 hr of light per day. Two groups were then transferred to DD and, 2 wk later, received 2 µl of a carbachol solution via the cannula at circadian time (CT) 22 or CT 14 (CT 12 = activity onset). One group was bilaterally enucleated and received carbachol at CT 22. Animals received an injection of a different concentration--between 0 and .03 molar--every 2 wk. Running-wheel activity was recorded throughout the study and plotted to determine the size of the phase shift induced by each injection. The table below shows the mean phase shift ± S.E.M., in hours, obtained at each dose. Injections at CT 22 induced phase advances and at CT 14 induced phase delays.

| | CONCENTRATION (moles/liter) | | | | | |
|---|---|---|---|---|---|---|
| | vehicle | .001 | .003 | .0056 | .01 | .03 |
| CT 22 | .2 ± .1 (N = 5) | .3 ± .1 (8) | .7 ± .2 (6) | 2.6 ± 1.0 (10) | 4.5 ± .5 (10) | 4.7 ± .8 (11) |
| CT 14 | | | .2 ± .1 (11) | .4 ± .1 (10) | .9 ± .2 (9) | |
| CT 22 (BL) | | | .1 ± .1 (9) | .1 ± .1 (9) | 1.0 ± .4 (8) | |

The magnitude of phase advance induced by a carbachol injection at CT 22 in hamsters exposed to DD appears to be dose-dependent, with a maximal response obtained at concentrations of .01 M or greater. However, the intermediate response obtained at .0056 M represents the mean of animals showing either a small or a large phase advance; only 2 of 10 animals actually showed an intermediate size phase advance. Although a complete dose-response curve for the other two groups has not yet been obtained, it appears that the magnitude of phase delay induced by carbachol at CT 14 is also dose-dependent, and that blind (BL) hamsters may be less sensitive to carbachol at CT 22 than sighted animals exposed to DD.

332.9 **DIAZEPAM BLOCKS LIGHT INDUCED PHASE ADVANCES OF THE CIRCADIAN LOCOMOTOR RHYTHM OF THE GOLDEN HAMSTER.** **M. R. Ralph and M. Menaker. Institute of Neuroscience, Dept. of Biol., University of Oregon, Eugene, OR 97403.**

**We have reported previously that light-induced phase delays but not phase advances of the circadian locomotor rhythm of hamsters can be blocked by the γ-aminobutyric acid (GABA) antagonist, bicuculline (Ralph and Menaker, 1985 Brain Res. 325:362). This suggested that GABA neurotransmission may be required for the processing of some, but not all light input to the clock. We have found that the benzodiazepine, diazepam, which can potentiate GABA transmission, blocks the effect of phase advancing light pulses on the hamster locomotor rhythm, but does not block the effect of phase delaying pulses.**

**Male golden hamsters (8-12 weeks of age), housed individually in running wheel cages in light tight boxes, were entrained to a 14:10 LD cycle for 7 days, and then released into constant darkness (DD). After 7 days in DD, each animal was given a 15 minute pulse of monochromatic light (515 nm; total fluence = 3.0 x $10^{14}$ photons·$cm^{-2}$·$sr^{-1}$) calculated to produce half maximal phase shifts at either phase advance (CT 18) or phase delay (CT 13.5) time points. At both time points, experimental animals received an i.p. injection of diazepam 30 minutes prior to the light pulse. Control animals received either saline or vehicle injections.**

**Diazepam blocked the effect of phase advancing light pulses (CT 18) but had no significant effect on phase delaying pulses (CT 13.5). The mean phase shifts induced by light in control groups were +1.02 ± .09 hr. and −.44 ± .10 hr. at CT 18 and CT 13.5, respectively. The blockade of light induced phase advances was dose dependent with an $ED_{50}$ of 7.5 mg/kg, and phase advances were completely blocked at doses above 12.5 mg/kg. Control injections of diazepam without light caused small phase delays (<0.2 hours), but these were not large enough to account for the reduction in the size of light-induced phase advances.**

**Taken together with the effects of bicuculline previously reported, these results suggest that GABA neurotransmission may be required for the processing of light-induced phase delays but may interfere with the processing of phase advances. Preliminary results indicate that bicuculline (2.5 mg/kg) may partly reverse the blocking effect of high doses (20 mg/kg) but not low doses (5 mg/kg) of diazepam. This raises the possibility that the effect of diazepam on phase advances may be mediated by more than one mechanism. Nonetheless, the data are consistent with the hypothesis that different neurochemical pathways may be involved in mediating light-induced phase advances and delays, and that GABA may be involved in one of those pathways.**

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332.10 ULTRADIAN ACTIVITY RHYTHMS IN PREWEANLING RATS: ATTENUATED BY DESIPRAMINE BUT NOT ZIMELIDINE. Martin H. Teicher and Ross J. Baldessarini. Department of Psychiatry and Neuroscience Program, Harvard Medical School: Mailman Research Center, McLean Hospital, Belmont, MA 02178.

Richter (1927) first described 1-2 h activity rhythms in developing rats. These are maximal at 15 d and recede with maturation (Teicher & Flaum: Dev Psychobiol 12:411, 1979). Ultradian motility rhythms with 1-2 h periodicity also occur in human infants and in the young of most mammals. These rhythms can reemerge in adults under certain pathological conditions (in preparation). The present study acsertained if such rhythms are altered by antidepressants.

Developing rats reared in litters of 10-12 were studied at 15-16 d of age, after IP administration of desipramine (DMI) or zimelidine (ZMI), chosen as selective inhibitors of uptake of norepinephrine (NE) and serotonin (5HT), respectively. DMI was administered at 4 and 20 mg/kg, and ZMI given at equimolar doses (5.25 and 26.4 mg/kg). At 30 min after treatment, rats were placed alone in dark isolation chambers without food or water for 24 h. Activity was recorded continuously with a 6-channel, computer-interfaced electronic activity monitor, and individual activity scores were recorded on disks at 10 min intervals.

Time-series data were analyzed by low-resolution variance spectral analysis, as recommended by Kripke (Adv Sleep Res 1:374, 1974). This procedure yielded the percent of total spectral variance in harmonic bands from 0 to 72 cycles/day (cpd) at 1 cpd increments.

Saline-treated control rats (n=18) displayed a broad ultradian peak at about 7-15 cpd (31% of total spectral variance), as well as a moderate 1 cpd (circadian) peak (3.4% of variance). The low dose of DMI (n=9) diminished ultradian rhythmicity in the 9-16 cpd band (35% mean reduction, p<.0001) and enhanced the circadian peak by 39% (p<.05); the high dose diminished ultradian rhythmicity at 8-17 cpd even more, by 40% (p<.0001) and enhanced the circadian peak greatly (180%, p<.001). ZMI (n=6), in contrast, had only small and inconsistent effects on ultradian rhythmicity and tended to reduce the circadian component.

These results suggest that the balance between circadian and ultradian modulation of activity can be shifted by some antidepressants in a direction found with normal maturation. The selective NE-enhancer, DMI, increased circadian cyclicity at the expense of ultradian rhythms in young rats in a dose-dependent manner, while the 5HT-uptake inhibitor ZMI did not share this effect and may have had opposite actions to reduce circadian rhythmicity.

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333.1 **Ontogeny of Thyrotropin-releasing hormone (TRH) metabolism in human cerebrospinal fluid (CSF).** **J.K. Rao*, E. Ponte *, A. Lopez*, A. Jayaraman and C. Prasad.** Departments of Medicine, Pediatrics, and Neurology, Louisiana State University Medical Center, New Orleans, La. 70112.

In tissue extracts, TRH is metabolized by two separate pathways, one intiated by an amidase (forming acid TRH, pGlu-His-Pro) and the other by pyroglutamate aminopeptidase (pGlu-peptidase). Cyclo (His-Pro), the product of the second pathway, has been shown to exhibit a variety of biologic activities in both man and animals. We have recently shown that TRH metabolism in human CSF is almost exclusively catalyzed by pGlu-peptidase. Since CSF is in constant exchange with the brain extracellular fluids, studies on the ontogeny of TRH metabolism in CSF might give insight into the functions of TRH in the brain.

[$^3$H-Pro]-TRH (20µM, 0.1µCi)was incubated at 37°C for 15, 30, 45 and 60 min. and the rates of synthesis of radioactive proline, acid TRH, and cyclo (His-Pro) was determined. Both amidase and pGlu-peptidase pathway activities were calculated by the rates of Pro + acid TRH and cyclo(His-Pro) formation respectively. The data presented below show that in CSF, TRH is exclusively metabolized via pGlu-peptidase pathway and the activity of this pathway in CSF from pre-term and newborn babies is considerabley lower($p<0.01$) than in adults.

| ONTOGENETIC STAGE | pmol / min / ml of CSF | |
|---|---|---|
| | pGlu-peptidase | amidase |
| Pre-term (29-36wks) | 2.29 ± 0.68(12) | < 1.0 (12) |
| Newborn (0-8days) | 4.05 ± 2.51 (8) | < 1.0 (8) |
| Adult (20-40years) | 19.20 ± 1.67 (7) | 1.02 ± 0.20(7) |

The observed low levels of pGlu-peptidase activity in pediatric CSF was not due to presence of enzyme inhibitors because, i) the enzyme activity did not increase following concentration by salt precipitation and dialysis (undialyzed: 2.62±1.02, n=5; dialized: 1.54 ± 0.47 pmol/min/ml, n=5; $p>0.2$), ii) the addition of increasing amounts of pediatric CSF in assay mixture did not affect the enzyme activity. Conclusions: 1) pGlu-peptidase is the major pathway of TRH metabolism in human CSF. 2) Very low activity of pGlu-peptidase in CSF from pre-term and newborn babies suggests a unique developmental role of this enzyme in brain TRH function(s).

333.2 ONTOGENY OF HYPOTHALAMIC CATECHOL AND INDOLEAMINES AS MEASURED BY HPLC. O. Khorram*, T.H. Le*, S.M. McCann, L. Krulich* (SPON: J. Lipton) Dept. of Physiology, Univ of Tex Hlth Sci Ctr, Dallas, TX.

The hypothalamic content of norepinephrine (NE), dopamine (DA), 5-hydroxyindoleacetic acid (5HIAA), 5-hydroxytryptamine (5HT), and 5-hydroxytryptophan (5HTP) were measured in rats starting on day 17 of prenatal life and continuing until day 34 of postnatal period. The amines were measured by HPLC with electrochemical detection. Prenatally, there was a steady increase in the NE levels reaching peak levels just prior to delivery. This increase was followed by a decline on the day of birth. Postnatally NE levels increased progressively with age, not reaching adult levels as late as day 34. Starting on day 20 of postnatal period a sex difference in NE levels was found with females having significantly higher levels than males. This sex difference was evident as late as day 34. Hypothalamic DA levels were low and showed no significant changes prenatally. The first increase in the levels of DA was seen on day 5. 20 day old females had significantly higher DA levels as compared to males. This sex difference was not evident on later days. α-methyl-p-tyrosine (α-MT)(150 mg/kg, i.p.) had no effect on NE levels in 10 day old rats, whereas it induced a significant decline in DA levels. The same results were obtained in 15 day old animals using a higher dose (200 mg) of α-MT, except that a slight decrease in NE levels was found 2 hours after the drug injection.

5-HT levels increased progressively pre and postnatally. 20 and 24 day old rats had higher 5-HT levels than males. High concentrations of 5-HIAA were measurable in fetal hypothalami with major increases before delivery, and on the day of birth. Postnatally no major changes in 5-HIAA levels were found, although 20 and 24 day old females had higher levels of 5-HIAA as compared to males. Contrary to the adult rat which has no detectable levels of 5-HTP, this indoleamine metabolite could be detected as early as day 17 of fetal period. 5-HTP concentrations peaked just prior to delivery and showed no significant changes postnatally. Injection of m-hydroxybenzyl hydrazine (100 mg/kg, i.p.) produced a significant increase in the levels of 5-HT and 5-HIAA in 15 day old rats, suggesting a high turnover of indoleamines is present early on in life.

Unknown peaks not corresponding to any known amine metabolite and absent from adult hypothalmi were found in both the fetal and postnatal period. Further investigation of amine metabolism during development and identification of these unknown peaks will be valuable in understanding hypothalamic regulation of pituitary function. Supported by NIH grants HD 09988, HD-07062.

333.3 **NEUROTRANSMITTER SYNTHESIS, STORAGE, AND TURNOVER IN NEONATALLY DEAFFERENTED SYMPATHETIC NEURONS.** A.J. Smolen and P. Beaston-Wimmer*. Dept. of Anatomy, Med. Coll. of Pa./E.P.P.I. Division, Philadelphia, PA. 19129.

Transsynaptic influences are thought to play a critical role in the developmental regulation of the expression of neurotransmitter related characteristics. In the superior cervical sympathetic ganglion (SCG) of rodents, tyrosine hydroxylase (TH), the rate limiting enzyme in catecholamine biosynthesis, undergoes a normal postnatal increase in activity, and this increase in activity fails to occur when the afferent input to the neurons of the SCG is removed at the time of birth. It is clear that the metabolism of neurotransmitter in a neuron does not depend solely on the activity of the synthetic machinery. Other factors which must be considered include the rate of release of the transmitter from the nerve terminals, the rate of reuptake, and the rate of degradation of transmitter within the cell. In the present study, we have begun to examine the metabolism of NE in sympathetic neurons and its regulation by transsynaptic influences by measuring the content and turnover of NE in SCG neurons. NE content was measured using high performance liquid chromatography with electrochemical detection, and the turnover of NE was determined by measuring NE content 3 hours after treatment with alpha-methyl- paratyrosine, which inhibits TH activity. NE content in the SCG underwent an 8 fold increase from the time of birth, and stabilized at adult levels at one month. Following neonatal deafferentation, there was a temporary stunting of NE accumulation. When regeneration of the afferents was permitted, there was a permanent reduction in TH activity, although the amount of NE increased to normal levels. Turnover of NE also reached normal levels. When regeneration of the afferents was prevented, TH activity was again permanently reduced, while the amount of stored NE increased to 70% of control levels. In this case, turnover of NE was significantly reduced. We conclude that the normal turnover of NE following reinnervation is most likely due to a return of synaptic activity impinging on the SCG neurons and a return of release of neurotransmitter from the nerve endings of these cells. These data suggest that there is a critical period during the first several postnatal weeks when afferent influences must be present in order for TH to undergo its normal increase in activity. By contrast, the amount of NE stored by sympathetic neurons and the rate of turnover of NE is not subject to the same critical period as is TH, but appears to be dependent on the activity of the neuron.

333.4 GLUCOCORTICOIDS AFFECT PROLIFERATION OF SMALL INTENSELY FLUORESCENT (SIF) CELLS IN SUPERIOR CERVICAL GANGLION (SCG). M.C. Bohn, Dept. of Neurobiology and Behavior, State University of New York, Stony Brook, New York 11794.

Dexamethasone (DEX) treatment of newborn rats increases the number of SIF cells in the SCG. These calls can be visualized by their intense catecholamine fluorescence and staining for tyrosine hydroxylase immunoreactivity (TH-IR). In addition, many SIF cells are stained for phenylethanolamine N-methyltransferase (PNMT) in DEX treated rats, but not in normal rats. Previous studies have shown that SIF cell responses to DEX are limited to the first postnatal week. The processes leading to an increased SIF cell number following DEX and loss of SIF cell responsiveness are presently unknown. This study was undertaken to determine whether SIF cell precursors are dividing in the postnatal rat and, if so, how DEX affects proliferation of these cells.

To determine whether division normally occurs in SIF cell precursors postnatally, rats were sacrificed two hours after an injection of 3H-thymidine (3H-T) on postnatal days 0,2,5,7,10,14 or 20. SCG sections were stained for TH-IR and processed for autoradiography. Many SIF-like cells were covered with silver grains in rats receiving 3H-T during the first postnatal week, but not thereafter.

The effect of DEX on SIF cell division was determined similarly by comparing the labeling index of SIF cells on postnatal day 6 in control and DEX (0.1µg/g; days 0-5) treated rats injected with 3H-T on days 0,2,4 or 6. The labeling index was markedly depressed in the DEX treated group even though many more SIF cells were observed. Labeled PNMT-IR cells were also observed.

These observations suggest that SIF cells are being generated during the first postnatal week in rat and that high levels of catecholamine phenotypic characters are present in dividing precursor cells. They also suggest that DEX inhibits proliferation of these precursors. Consequently, glucocorticoids appear to enhance the level of catecholamine characters in pre-existing cells, rather than stimulate proliferation of SIF cell precursors.

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333.5 GLUCOCORTICOID HORMONE EFFECTS ON TRANSIENTLY CATECHOLAMINERGIC CELLS OF EMBRYONIC RAT INTESTINE STUDIED IN VIVO AND IN VITRO. G. Miller Jonakait and I.B. Black, Division of Developmental Neurology, Cornell Univ. Med. Coll., New York, N.Y. 10021.

Cells which transiently express catecholaminergic characteristics have been described in the embryonic rat intestine. Cells displaying immunoreactivity to tyrosine hydroxylase (T-OH) and formaldehyde-induced fluorescence (FIF) appear at 11.5 days of gestation (E11.5), but are no longer detected after E14. However, treatment of pregnant rats with reserpine on E11.5 results in 1) increased T-OH enzymatic activity in 13.5-day guts and 2) prolonged expression of T-OH and FIF in fetal gut perikarya (Jonakait et al., 1982). This paradoxical action of reserpine is probably mediated via maternal glucocorticoid hormones (GCH) since 1) maternal cortisol treatment mimics the effect of reserpine and 2) compromise of the maternal adrenal cortex with mitotane diminishes the effect of reserpine treatment. We now report that maternal drug regimens designed to suppress reserpine-induced increases in plasma GCH also block the reserpine-induced increases in fetal enteric T-OH activity. Thus, treatment of pregnant rats with either the synthetic GCH dexamethasone (dex; .1 mg/kg) or pargyline (25 mg/kg) 1 or 2 days prior to reserpine treatment blocked increased plasma GCH and also resulted in normal T-OH activity in E13.5 guts. These data confirm that the effects of reserpine on the fetal intestine involve increases in maternal GCH.

To determine whether GCH affect noradrenergic traits other than T-OH and FIF, we measured the high-affinity uptake of $^{3}$H-norepinephrine into T-OH-positive cells of the intestine at E13.5 (Jonakait, et al., 1985), but found it to be normal following reserpine treatment. This finding suggests that different characters of the noradrenergic phenotype are, in this instance, regulated differently.

We sought next to examine the site of GCH action. Dex, administered into the fetal environment via a transuterine injection at E11.5, had no demonstrable effect on T-OH activity measured at E13.5. However, to eliminate the possible confounding effects of placenta and fetus, we grew isolated E12.5 intestines in organotypic tissue culture. The medium contained Eagles' Minimum Essential Medium and 15% rat serum taken either from normal or adrenalectomized female rats. Growth in adrenalectomized rat serum or in medium supplemented by dex, cortisol, corticosterone, or cortexolone (0.1-10 uM) had no effect on T-OH activity measured after 4 days in vitro. Therefore, while the action of reserpine cannot occur without maternal GCH elevation, other factors or processes may be necessary for steroid action at the level of the fetal intestine. (Supported by NIH grant NS 17814).

333.6 DEPOLARIZING SIGNALS INCREASE TYROSINE HYDROXYLASE DEVELOPMENT IN CULTURED MOUSE SUBSTANTIA NIGRA. W.J. Friedman, C.F. Dreyfus, B.S. McEwen and I.B. Black. Rockefeller Univ. and Cornell Univ. Med. Coll., N.Y., N.Y. 10021.

While extracellular signals are well known to regulate neurotransmitter development in the peripheral nervous system, factors governing brain development remain to be characterized. Development of the dopaminergic phenotype in the mouse substantia nigra (SN) was studied by monitoring tyrosine hydroxylase (TH). This enzyme catalyzes the initial, and rate-limiting, step in catecholamine biosynthesis. Ventral mesencephalic explants were dissected from E15 mouse embryos and maintained in culture for one week. To define the effect of depolarizing influences on central neurons, cultures were grown with the pharmacologic depolarizing agent veratridine. This treatment elicited a significant increase in development of TH activity. Since the nigra receives an excitatory substance P innervation from the striatum, the effect of a stable analogue of the peptide (pGlu$^{5}$,MePhe$^{8}$,Sar$^{9}$) was examined. The presynaptic agonist elicited a significant increase in TH activity, reproducing the effects of pharmacologic stimulation.

To determine whether the rise in TH catalytic activity evoked by veratridine and substance P reflected elevation of enzyme protein, Western transfer analysis was employed. Immunoblots probed with anti-TH antiserum and analyzed densitometrically exhibited increased amounts of enzyme protein.

Our observations indicate that depolarization with a pharmacologic agent or with a presynaptic agonist elicit increased TH activity as well as enzyme molecule number. Studies are in progress to investigate underlying molecular genetic mechanisms governing these phenomena. (Supported by NIH grants NS 29788, HD 12108, NS 10259, NSF grant 80 24081, and the American Parkinson Disease Association. C.F.D. is the recipient of a Teacher-Scientist Award from the Andrew W. Mellon Fdn. )

333.7 EXTRACELLULAR FACTORS REGULATE NEUROTRANSMITTER PLASTICITY IN MATURE SYMPATHETIC NEURONS. D.R. Kornack, J.E. Adler AND I.B. Black. Div. of Developmental Neurology, Cornell Univ. Med.Coll., New York, NY 10021.

Abundant evidence suggests that developing neurons are remarkably plastic, altering transmitter phenotypic expression in response to extracellular stimuli. To determine whether transmitter plasticity persists during maturity, we have examined mutability of peptidergic and catecholaminergic transmitter traits in adult rat superior cervical ganglia in vivo and in vitro.

Ganglionic decentralization (denervation) in adults increased substance P (SP) 4-fold in vivo. To examine mechanisms underlying this increase, adult ganglia were explanted to culture in the absence of added nerve growth factor (NGF). Explantation resulted in a 4-fold rise of SP in vitro, reproducing the results obtained in vivo. Veratridine prevented the increase in cultured adult ganglia, while tetrodotoxin blocked the veratridine effect, suggesting that membrane depolarization and sodium influx normally suppress SP content in mature ganglia, as in neonatal ganglia. Since NGF is well-known to govern sympathetic development, we examined the response of adult explants to the trophic agent. NGF exposure increased SP 15-fold in adult ganglia, suggesting that transmitter metabolism in mature sympathetic neurons remains responsive to the trophic protein. To determine whether peptidergic and catecholaminergic transmitter traits are differentially regulated in mature ganglia as in developing ganglia, we examined the activity of tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine biosynthesis. Explantation did not increase TH activity in adult ganglia, reproducing results previously obtained in the neonate.

Our observations suggest that remarkable plasticity persists in mature neurons in vivo and in culture. Further, adult sympathetic neurons remain responsive to NGF in vitro. Finally, mature neurons differentially regulate different transmitter phenotypic traits. (Supported by NIH Grants NS 10259, HD 12108 and aided by a Grant from the Hereditary Disease Foundation).

333.8 DEPOLARIZATION AND CALCIUM IONS REGULATE THE LEVEL OF ADRENAL PREPROENKEPHALIN mRNA. E.F. LaGamma, J.D. White, J.E. Adler, J.E. Krause, J.F. McKelvy, and I.B. Black. Cornell Univ. Med. Coll., N.Y. 10021 and SUNY, Stony Brook, N.Y. 11794.

To begin defining the molecular processes through which extracellular signals regulate co-localized transmitters, rat adrenal medullae were grown in explant culture. The (now denervated) explanted medullae exhibited a 10-fold increase in leucine-enkephalin (LEU) after 3 days in culture. Inhibition of protein synthesis with cycloheximide completely blocked the rise, whereas, inhibition of RNA synthesis with actinomycin-D, α-amanitin, or camptothecin inhibited the increase by 50%. To determine whether the rise in LEU was associated with an increase in specific mRNA coding for the opiate peptide precursor, northern blot analysis was performed using a $^{32}$P nick-translated cDNA probe complementary to human pheochromocytoma preproenkephalin mRNA (generosity of Dr. E. Herbert). A single species of preproenkephalin mRNA was detected after varying culture periods. The mRNA increased 34-fold after 2 days in culture and 74-fold after 4 days. Consequently, the rise in mRNA preceded the increase in LEU. Depolarization with either elevated potassium or veratridine, which prevents LEU accumulation, inhibited the increase in preproenkephalin mRNA as well. Moreover, the effect of veratridine was blocked by tetrodotoxin, suggesting that trans-membrane sodium ion influx affects elaboration of the message. To determine whether the depolarization effect was calcium ion-dependent, medullae were depolarized in the presence of EGTA or in the presence of a calcium ion "channel" blocker (D600). Inhibition of $Ca^{2+}$ influx prevented the effects of KCl-induced depolarization on LEU. Increasing intracellular $Ca^{2+}$ with the ionophore A23187, in the absence of depolarizing agents, reproduced the effects of depolarization, inhibiting the rise in LEU. Our studies suggest that depolarization, $Na^{+}$ and $Ca^{2+}$ influx affect levels of preproenkephalin mRNA. Further, in conjunction with the metabolic inhibitor data, our observations suggest that extracellular signals causing depolarization may alter gene read out. The effects of $Ca^{2+}$ on preproenkephalin mRNA is presently under investigation. (Supported by NIH Grants HL 00756, NS 10259, HD 12108 and NSF-BNS 8111475, NS 20372.

334.1 GENESIS OF CONCURRENT ASYNCHRONOUS PERIODIC RESPIRATORY PATTERNS. G.P. Moore and D. Hary*. Biomedical Engineering, University of Southern California, Los Angeles, California 90089-1451.

Under special circumstances, it is possible to induce a human subject to generate multiple, concurrent, asynchronous, periodic breathing patterns. Subjects were fitted with a face mask, allowing continuous recording of inspiratory and expiratory airflows and pressures. While testing the respiratory patterns of subjects who were instructed to breathe in synchrony with a visual pacing signal (whose rate, and inspiratory/expiratory duty cycle could be independently adjusted), we accidently discovered that after the subject successfully synchronizes for several minutes with the pacing signal, the parameters of that signal can be altered in such a way that, while he consciously follows the new pattern of the pacing signal, he may also unknowingly continue to breathe, concurrently, as if the previous pacing signal pattern were still present. Over a period of several minutes the subject continues to generate, although at a reduced amplitude, the periodic pattern of flow and pressure previously employed, while also generating a pressure-flow pattern conforming to the timing constraint of the new pacing signal. The initial, mutually asynchronous patterns eventually achieve an alternating phase-locked relationship, the previous pattern gradually shifting its timing to match the period of the current pacing signal. During the transition period, the onset-times of inspiration for the two generators may coincide. Yet, thereafter, the two patterns re-emerge and continue without interruption. When the onset-time of inspiration of one generator cycle drifts into the expiratory phase of the other generator cycle, monitored EMG signals of respiratory muscles show that the inspiratory generator immediately increases its output to compensate for, and effectively override, the expiratory effect of the other generator. This latter observation implies that a human can not only generate independent, concurrent respiratory patterns, but that these generators may ultimately compete at the level of the final common pathway. The flow and pressure patterns clearly indicate that, under these circumstances, what we might otherwise reasonably define as inspiration and expiration can occur simultaneously.

334.2 PHRENIC NERVE PERIODICITIES IN NEONATAL SWINE. H.L. Cohen, P.M. Gootman, A.M. Steele*, M.R. Gandhi*, L.P. Eberle* and P.P. Rao*. Dept of Physiology, Downstate Medical Center, SUNY, Brooklyn, N.Y. 11203 and Schneider Children's Hospital of Long Island Jewish-Medical Center, New Hyde Park, New York 11042.

Power spectral analyses of efferent phrenic (PHR) nerve discharge were used to assess maturational changes in the central respiratory oscillator of neonatal swine. Piglets from < 1 day to 39 days were anesthetized with Althesin ( 4 mg/kg/hr), immobilized with C10 (decamethonium Br), maintained on 100% 02 and artificially ventilated. Monophasic recordings were made on magnetic tape of left and/or right PHR activity (bandpass 3-10k Hz), integrated PHR activity, intratracheal pressure (ITP), blood pressure, EKG and markers indicating phases of the artificial ventilatory cycle. Power spectral analyses were performed on a PDP 11/45 computer. Sampling was triggered during inspiration (I = onset of PHR burst). Power spectral densities of PHR activity were investigated at a sampling interval of 244 μsec and 1024-2048 points/breath using the Fast Fourier Transform (FFT). Typically, two 1024 data points FFTs were computed per 500 msec epoch. The Nyquist foldover rate determined the low pass filter setting of 1 kHz. Average power was computed from 100-200 epochs sampled during I. Hanning windows were applied to reduce side lobe leakage. Results were plotted as average power per frequency interval (2 Hz). Examination of the power spectral peaks indicated that predominant activity occurred in two frequency bands: 1) a low frequency discharge from 15-35 Hz, which appeared to occur more frequently in younger piglets (< 2 weeks of age) apparently modulating a higher frequency discharge and 2) the higher frequency discharge, which ranged from 100-150 Hz and was an increasing function of age ($r = .88$). Two predominant peaks have also been reported for adult cats (Richardson, C.A. & Mitchell, R.A., Brain Res., 233: 317, 1982). Furthermore, we found that vagotomy did not produce a shift in peak power at the higher frequencies. Our results indicate that power spectral peaks in the range of 100-150 Hz can be used to monitor maturation of the central respiratory oscillator.
(Supported by NIH Grant #HL-20864).

334.3 A PROJECTION FROM A DISCRETELY LOCALIZED CELL GROUP NEAR THE VENTRAL SURFACE OF THE ROSTRAL MEDULLA TO THE VENTRAL RESPIRATORY GROUP (VRG) IN THE CAT. H.H. Ellenberger*, J.C. Smith*, D.R. McCrimmon* and J.L. Feldman. (SPON: A. Gibson) Department of Physiology, Northwestern University , Chicago, IL 60611.

Neurons with somas in the VRG of the cat medulla provide the respiratory drive to phrenic motoneurons. The source of the phasic respiratory activity of VRG neurons has yet to be determined. It is possible that phasic inputs from a neural oscillator and inputs from central chemoreceptive structures arise from outside the VRG. To investigate these possibilities, projections to the VRG were examined using 1% wheat germ agglutinin conjugated horseradish peroxidase (HRP) as a retrograde tracer. Tissue was processed with a sensitive reaction using tetramethyl benzidine as the chromagen (Gibson et al, Brain Res., 298: 235, 1984). Pressure injections of (8-20nl) HRP solutions were made using a glass pipette fitted with a silver wire so that single-unit activity could be recorded prior to injection. Injections were centered within regions of phasic inspiratory cell activity in the VRG. In addition to observing projections described previously (Bystrzycka,Brain Res., 185: 59,1980), a novel projection to the VRG was identified. This group of cells is located near the rostral medullary ventral surface near the caudal end of the trapezoid nucleus. The cells of this compact group, termed here the retrotrapezoid nucleus (RTN) have small (~20um diameter), mostly fusiform somas positioned 200-300um deep to the ventral surface and about 3.5mm lateral to the midline. The rostrocaudal length of RTN is about 1.5mm. The RTN begins caudally as a small cluster of cells below the ventromedial tip of the most caudal portion of the facial nucleus, immediately ventrolateral to the nucleus paragigantocellularis lateralis. Further rostrally, cells of the RTN increase in number as the nucleus broadens in the lateral dimension beneath the facial nucleus. Rostrally, the RTN is bounded by the preolivary nucleus. The RTN projection to the region of the VRG appears to be exclusively ipsilateral. A respiratory function for the RTN remains speculative. Its superficial position in the rostral medulla suggests that these cells may be important in central chemosensitivity, perhaps providing the link between central chemosensitive structures and the inspiratory cells of the VRG. The RTN appears to lie in the rostral portion of the "intermediate chemosensitive area" of the ventral medullary surface, where cold block produces apnea in vagotomized cats (von Euler et al, in: Neurogenesis of Central Respiratory Rhythm,45,1985). An RTN projection to neurons in the VRG could mediate the effects of this chemosensitive region on phrenic nerve activity. Supported by NIH HL-23820 & T32-NS07243.

334.4 EFFECT OF NALOXONE ON THE CENTRAL RESPIRATORY CHEMOSENSITIVE ZONES OF THE CAT. J. A. Whittaker, D. G. Bernard, P. W. Archer, C. O. Trouth., Department of Physiology & Biophysics, Howard University, Washington, D. C. 20059.

Three chemosensitive zones on the ventrolateral surface of the medulla oblongata (VMS) are involved in the central chemical $CO_2$ regulation of respiration; a rostral, and intermediate and a caudal; the caudal area being the most sensitive to chemical and electrical stimulation. In spontaneously breathing cats anesthetized with alpha-chloralose (60 mg/kg), neuron in the caudal area were electrophysiologically tested for their responsiveness to topically applied acetylcholine (ACh), morphine, and naloxone, as well as to pH changes (7.4 - control, 7.0 - acid; 7.8 alkaline) following superfusion with mock cerebrospinal fluid (CSF). Topical application was by means of filter paper pledgets (1 mm $^2$) soaked in the test drugs that were prepared in mock cerebrospinal fluid (CSF) at pH 7.4. Units were also tested for their responsiveness to other modalities of sensation such as touch, pressure, joint manipulation and temperature. Non-phasic pH sensitive units increased their firing rate in response to increased inspired $CO_2$ (5% and 7%), CSF-pH 7.0, topical application of ACh (10 mg%) and the opiate antagonist naloxone (2 mg%). CSF-pH 7.8 and morphine (2 mg%) decreased neuronal firing rate from 23 to 6 Hz. Tidal volume and respiratory frequency which were recorded spirometrically in 3 cats also decreased following topical morphine application and/or superfusion with alkaline CSF. 2 mg% naloxone reversed the effects of alkaline (pH 7.8) superfusion as well as topical morphine application. Naloxone potentiated the effects of increased inspired $CO_2$ as well as topically applied ACh and acid superfusion (pH 7.1) causing marked increases in firing rate.

It appears that the endogenous opioids are involved in modulation or fine-tuning of the neuronal activity of non-phasic-pH sensitive neurons in the caudal chemosensitive areas of the cat.
Support: NIH - training grant 52 T 32 GM 07800 and NIH-MBRS Grant 2 S06RR08016.

334.5 ANTICHOLINESTERASE TOXICITY AT CENTRAL RESPIRATORY CHEMOSENSITIVE AREAS REDUCED BY OPIATE ANTAGONIST: P.W. Archer, R.M. Millis, J.A. Whittaker, D.G. Bernard, C.O. Trouth, Dept. Physiology & Biophysics College of Medicine, Howard Univ., Washington, D.C. 20059

Central respiratory chemosensitivity has been ascribed to neurons located within the marginal glia in caudal (Area L) and rostral (Area M) zones on the ventral medullary surface (VMS). In the present study the respiratory effects of cholinomimetics and opiates applied topically to the caudal area was investigated in cats anesthetized with α-chloralose (60 mg/kg). The drugs were topically applied by means of filter paper pledgets (1mm$^2$) soaked in the test drugs that were prepared in mock cerebrospinal fluid (CSF) at pH 7.4.Low concentrations of Acetylcholine (ACh) 2-8 mg% had no significant respiratory or cardiovascular effect. Graded increases in tidal volume (VT) and respiratory frequency (f) were observed at higher concentrations (8-25 mg%) however without cardiovascular effects.Nicotine (2-200 mg%) applied topically produced significant ($p<0.001$) increases in VT and f whereas mean arterial blood pressure (MABP) decreased significantly. Intravenous injections of Nicotine (200 mg) caused increases in VT, f and MABP. Physostigmine (2-20 mg%) potentiated the effects of ACh on respiration in dose-dependent manner, without cardiovascular effects. Larger concentrations of physostigmine (>25 mg%) produced systemic hypertension and tachycardia followed by apnea and hypotension. The cardiovascular effects of physostigmine were antagonized by atropine sulfate (AS) without affecting respiration. 8-25 mg% AS raised MABP and heart rate to control levels but apnea persisted. The opiate antagonist Naloxone (2-8 mg%) which caused marked increases in VT and f in spontaneously breathing cats, reversed the physostigmine induced apnea when topically applied. Morphine sulfate (2-20 mg%) at low concentrations decreased VT and f without significant vasomotor effects, however, at higher concentrations it caused apnea hypotension and bradycardia. Naloxone reversed the morphine induced respiratory depression and/or apnea whereas physostigmine at low doses (2-20 mg%) potentiated the respiratory effects of morphine.

Conclusion 1) Since acetylcholinesterase hydrolyzes not only ACh but also some Peptides - among them the endogenous opiates (EO)[1] it appears that the effect of topically applied physostigmine to the chemosensitive structures on the VMS may be partly due to its action on the EO system at this site. A modulatory role of the endogenous opiate system in the central chemosensitive drive to respiration is postulated. 2) The opiate antagonist Naloxone reduces the respiratory effects of reversible anticholinesterase toxicity of the central respiratory chemosensory apparatus.

[1]Chubb, et al: Neurosci: 10,1369-1377, 1983. Supported by: NIH-MBRS2-S06RR08016.

334.6 INDUCED TRANSIENT HYPERTENSION INHIBITS LARYNGEAL ABDUCTOR ACTIVITY IN THE DRUG-FREE CAT. J.D. Marks, R.C. Frysinger, and R.M. Harper. Brain Research Institute and Department of Anatomy, UCLA, Los Angeles, CA 90024.

Cardiovascular dynamics affect diaphragmatic and upper airway muscle activity. Transient hypertension following pressor agent infusion is accompanied by decreases in respiratory rate and in peak integrated diaphragmatic EMG. Under anesthesia, the suppression of phasic respiratory activity is greater in certain upper airway muscles (e.g., genioglossus) over the diaphragm. We examined how transient hypertension affects the laryngeal abductors of the upper airway in the drug-free cat.

Five adult female cats had electrodes surgically implanted under halothane-nitrous anesthesia. Teflon-coated, stainless-steel stranded wires were placed in the diaphragm and posterior cricoarytenoid (PCA) muscle for recording diaphragmatic and laryngeal abductor EMG. Polyethylene tubing was placed in the abdominal aorta via the femoral artery for recording blood pressure, and in the right atrium via the internal jugular vein for administering pressor agents. Electrodes for monitoring sleep-waking state were also implanted. EMG activity was amplified, band-pass filtered, rectified, and integrated. Data were recorded onto polygraph paper and were digitized by a PDP-11 minicomputer. Transient hypertension was induced with phenylephrine hydrochloride (10 ug/kg) in a 0.2 mL bolus of 0.9% NaCl during waking, quiet sleep, and REM sleep states. Sections of the record starting four breaths before pressor administration were extracted for analysis. Respiratory statistics were calculated by a computer using the integrated diaphragmatic and PCA traces, and were correlated with blood pressure on a breath-by-breath basis.

Following pressor administration, blood pressure rose 60-80 mm Hg. The respiratory rate fell during the first three breaths from the beginning of the pressor response, and expiratory duration increased. In both the diaphragm and PCA, peak integrated EMG decreased during the first three breaths in all three states. However, peak integrated EMG in PCA decreased much more from control values than in diaphragm, as did the total integrated EMG amplitude. Occasionally, PCA phasic EMG activity disappeared for several breaths following hypertension onset in the absence of diaphragmatic silence. When the diaphragm did become silent during transient hypertension, phasic PCA activity disappeared as well.

These results provide evidence that neural mechanisms underlying laryngeal abductor activity are more affected by inhibitory baroreceptor responses to transient hypertension than diaphragmatic control mechanisms.

Supported by HL 22418-08.

334.7 RATE DISCHARGE PATTERNS OF NEURONS IN THE CENTRAL NUCLEUS OF THE AMYGDALA: RELATIONSHIP WITH STATE AND RESPIRATORY ACTIVITY. J.X. Zhang, R.C. Frysinger, and R.M. Harper. Brain Research Institute and Department of Anatomy, UCLA, Los Angeles, CA 90024.

Neurons of the central nucleus of the amygdala (ACE) discharge phasically with the cardiac and respiratory cycle, and these relationships are state dependent (Zhang et al., Soc. Neurosci. Abs. 9: 1163, 1983). Moreover, cryoprobe blockade of the ACE decreases the amplitude of spontaneous diaphragmatic inspiratory efforts in the awake state, but this effect is greatly reduced by state. The state-dependent nature of these neuronal discharge characteristics led us to partition these cells by rate and location within the ACE, and to describe spontaneous discharge rates of neurons within this nucleus.

Bundles of fine wire microelectrodes, together with electrodes to record sleep-state and cardiorespiratory parameters, were placed in the ACE of four cats under halothane anesthesia. After surgical recovery, we recorded neuronal discharge, diaphragmatic EMG activity, ECG, and EEG from the intact, unrestrained, drug-free cat during waking (AW), quiet sleep (QS), and active sleep (REM) states.

Cell discharge rates in the ACE were low (n=207, range: <1/s to 90/s), with the greatest proportion (68%) discharging less than 10 spikes/s and 15% discharging less than 2 spikes/s. Cell discharge rate differed, depending on location within the ACE. The fastest firing cells (30-90 Hz) were located in the posterior dorsomedial region of the ACE. Most cells (n=71, 34%) discharged fastest in AW, slowest in QS, and faster in REM. The next most common pattern (16%) was rates slightly faster in QS than AW and faster in REM than QS. No consistent relationship between rate of discharge or change in rate of discharge across states and phasic discharge to the respiratory cycle was observed.

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334.8 PERIPHERAL AND CENTRAL CONTROL MECHANISMS DURING RESPIRATORY SUSPENSIONS IN TRANSCENDENTAL MEDITATION AS EVIDENCED BY LATENCY, HYPOXIA AND RQ CHANGE. J. Kesterson*, N. Clinch*(SPON:K. Walton). Dept. of Neuroscience, Maharishi International University, Fairfield, Iowa 52556.

Previous studies have reported the occurence of respiratory suspensions (RS) during Transcendental Meditation (TM) (Farrow, J., Psychosom. Med.,(44)2:133,1982). The current research attempts to explain the control mechanisms involved and their relationship to the previously reported decrease in metabolic rate associated with TM. Current findings include:

1) Six practitioners of TM (4 male) with consistent patterns of RS during TM were tested repeatedly (2 to 8 times) in a rest, TM, rest protocol. Metabolic rates and frequency of breathing were measured with Beckman gas analyzers. Metabolic rates dropped 5% to 25%. Sixteen suspensions from 10 to 45 seconds began within 5 seconds of the cue to begin TM, before metabolic rates began to drop.

2) A single male subject with consistent RS patterns during TM was tested for hypoxic and hypercapnic drives using a Stead-Wells spirometer and Beckman gas analyzers. The protocol was 10 minutes rest, 10 minutes TM. During TM,suspensions of up to 20 seconds persisted even with an end tidal CO2 as high as 57mmHg, an unusual condition considering the increase in frequency and tidal volume that results from such a high value of CO2. During rest, an end tidal O2 of 49 mmHg had no effect on frequency or tidal volume, but during TM, following suspensions, an end tidal O2 of only 73 mmHg produced a change from 4 breaths per minute to 18 breaths per minute.

3) Repeated testing of 6 meditators showing consistent RS using Beckman gas analyzers in a rest, TM, rest protocol showed drops from .03 to .20 in RQ with TM and an overshoot above pre-TM rest levels of .05 to .15 during post-TM rest. One subject was tested by collecting expired air in collection bags. RQ for 10 minutes rest was .84; for 20 minutes TM: .60; for 10 minutes post-TM rest: .95. Metabolic rates, and thus O2 consumption declined for the TM period. The drop in RQ was due to a decrease in CO2 exhaled.

Conclusions: 1) Initiation of the RS during TM is neurogenic and centrally controlled. 2) Cessation of the RS is a consequence of blood gas levels, with an increased contribution of hypoxic drive. 3) Drop of metabolic rate does not initiate the RS but may contribute to its duration.

335.1 EFFECT OF NEWBORN RAT DENERVATION ON THE SKELETAL MUSCLE CREATINE KINASE ACTIVITY. O.C. Ramírez, L.A. Baiza* and M.A. Ibañez*. Dept. Biochemistry, C.I.E.A. and Natl. Sch. Biol. Sci., Polytech. Inst. México, D.F. 07000

The effect of muscle disuse after denervation on the levels and rates of synthesis of several glycolyic enzymes in mature chicken breast-"white" muscle resulted in a 50% reduction in the concentration of these enzymes 2 weeks after severing the nerves. Denervation for 6 weeks did not have a significant effect on the levels of creatine kinase (CK) activity from non-contractile breast muscle (J. Biol. Chem. 256:6423, 1981). Results from mammalian and amphibian skeletal muscle, in vivo and in vitro, indicate that continued contractile activity resulted in an increased activity of CK, 5' adenylic deaminase and adenylic kinase (Biochem. J. 103:207, 1967). To try to solve the discrepancy between authors who observe changes in activity of muscle CK and those who do not, we studied the effect that denervation at birth of rat fast and slow twitch muscles had on muscle growth and on the levels of CK activity. A fragment of thigh sciatic nerve was removed and, as a control, the contralateral extremity was kept intact. After 30 and 70 days, significant differences in weight values and in CK specific activity were found among experimental and control fast-extensor digitorum longus (EDL) and slow-soleus muscles. Denervation caused a larger decrease on the weight of soleus as compared with EDL. The weight of denervated slow and fast muscles were 15.4 ± 5.4% and 20.8 ± 3.8% of their respective unoperated controls at 30 days; denervated EDL was 18.4 ± 2.1% of its normal control at 70 days. Denervated soleus was extremely atrophied at 70 days.

Regarding CK specific activities, denervated soleus and EDL contained 13.6 ± 4.2% and 32.9 ± 7.6% of their respective controls at 30 days; denervated EDL showed 37.6 ± 2.7% of its control at 70 days. No CK activity increase was shown from 30 to 70 days in control soleus (41.9 ± 7.9 to 41.1 ± 5.8 units per mg protein). Whereas, in contrast to chick mature fast muscle, control and denervated EDL showed a similar proportional activity increase from 30 to 70 days post-denervation (CK 70/30 days ratios were 1.3 and 1.5 respectively).

It is concluded that species-and muscle-type specificity, as well as distinct levels of muscular maturation are responsible for the observed nerve-dependent or nerve-independent changes in CK activity and muscle growth or atrophy.

335.2 DIFFERENTIAL EFFECTS OF TENOTOMY ON THE MOLECULAR FORMS OF ACETYLCHOLINESTERASE IN FAST- AND SLOW-TWITCH SKELETAL MUSCLES OF YOUNG RATS. M.R. Emmerling* and E. Schultz* (Spon: G.J. Royce) Dept. of Anatomy, UW-Madison, Madison, WI 53706

The fast-twitch extensor digitorum longus (EDL) and gastrocnemius (GAS) and slow-twitch soleus (SOL) of 30-day old rats were tenotomized in order to study the effects of disuse on the expression of acetylcholinesterase (AChE) and its molecular forms. Animals were sacrificed on days 0, 3, 7 and 14 of tenotomy. Unoperated muscles of the contralateral hindlimbs were used as controls. On day 3, the cut ends of the tendons remain separate but began to reattach by day 7 and were completely attached and functional by day 14. AChE activity was 15-45% greater in tenotomized EDL and GAS than control until day 14 when it became slightly less. In contrast, SOL AChE activity was less than control on all days studied. Analysis of AChE forms showed that increases in AChE activity in tenotomized EDL and GAS were due primarily to 10S AChE. Reductions in 16S, 12S and 6/4S AChE accounted for the loss of activity in tenotomized SOL. Tenotomy appeared not to affect 10S AChE in SOL or 16S AChE in GAS or EDL. To determine whether or not the effects of tenotomy can occur in the absence of neural influences, the EDL and SOL were tenotomized and/or denervated for 3 days. Denervation of the EDL affected the 16S and 6/4S AChE forms but not 10S AChE. Tenotomy of the EDL increased 10S but produced no change in 16S and 6/4S. In the SOL, denervation decreased 16S and 12S AChE, but not 10S AChE or 6/4S AChE which was markedly reduced by tenotomy along with the 16S and 12S AChE forms. In the SOL the combined effects caused a greater loss of both AChE activity and 16S, 12S and 6/4S forms than either of the treatments alone. It also caused the loss of 10S AChE not previously seen. In contrast, denervation/tenotomy of the EDL increased AChE activity and, specifically, 10S AChE, but not to the level in tenotomized only EDL. The above results reveal that tenotomy produces changes in AChE and its forms unique to muscle type and unlike those caused by muscle denervation. The fact that tenotomy has an effect on AChE even after denervation suggests that the mechanism by which tenotomy affects AChE expression is separable from neural influences. A major effect of muscle tenotomy is the elimination of passive stretch. It is likely that the elimination or stretch is partly, if not principally, responsible for the effects of muscle tenotomy on AChE. It is particularly intriguing that 10S AChE in EDL and 6/4S AChE in SOL remain after both denervation and tenotomy because it suggests that influences beyond those examined help determine whether or not a muscle type possesses a particular form of AChE. (Funded by NSF grant PCM 8302348).

335.3 NERVE BLOCK WITH TETRODOTOXIN MIMICS AXOTOMY OF CAT MG MOTONEURONS. J.B. Munson, R.C. Foehring and G.W. Sypert. Depts. of Neuroscience and Neurosurgery, University of Florida College of Medicine, Gainesville, FL 32610.

This work tested whether conduction block of the intact MG nerve of cats would result in changes in MG motoneuron electrical properties similar to those which occur following axotomy. Conduction block of 17 days duration was produced by perfusion of the MG nerve with TTX (.25 µg/hour) using an osmotic pump implanted subcutaneously (Betz et al. J. Physiol. 303). To date only one animal has fulfilled our requirements of (i) MG muscle contraction in response to electrical stimulation of the MG nerve distal to but not proximal to the perfusion cuff and (ii) absence of fibrillations of the MG muscle at the time of the terminal experiment. Motoneuron electrical properties measured were rheobase ($I_{Rh}$), input resistance ($R_N$), conduction velocity (CV) and afterhyperpolarization half-decay time (AHP). Measurements were made of MG motoneurons in normal (MG-NML), 2-3 weeks-axotomized (MG-AXT) and TTX-perfused (MG-TTX) cats. As an internal control, properties of LG motoneurons not TTX-perfused were also measured in the MG-TTX experiments (LG-EXP) and compared with normal LG (LG-NML). The following data were obtained (values are means; number in parentheses is n):

| | $I_{Rh}$ (nA) | $R_N$ (Mohm) | CV (m/s) | AHP (ms) |
|---|---|---|---|---|
| MG-NML | 14 (147) | 1.0 (117) | 94 (111) | 28 (118) |
| MG-AXT | 4 (67) | 2.6 (60) | 70 (72) | 30 (65) |
| MG-TTX | 5 (21) | 2.1 (21) | 74 (28) | 26 (21) |
| LG-NML | 17 (102) | 1.0 (97) | 96 (99) | 23 (101) |
| LG-EXP | 16 (6) | 1.1 (6) | 88 (9) | 20 (6) |

These limited preliminary data from one successful experiment support the hypothesis that the electrical signs of motoneuron axotomy may also be produced by TTX-perfusion of the intact MG muscle nerve. Activation of the neuromuscular synapse may be one requirement for expression of normal, mature MG motoneuron properties (Czeh et al. J. Physiol. 281). (Supported by NS 15913 (NIH) and the MRS (VA).

335.4 DENERVATED FAST-TWITCH MUSCLES OF MICE: MYOTROPHIC EFFECTS OF NERVE EXTRACT. H.L. Davis, B.H. Bressler*, L.G. Jasch*, and E.A. Heinicke*. Departments of Anatomy; McGill University, Montreal, Canada; University of British Columbia, Vancouver, Canada; University of Western Ontario, London, Canada.

Changes in a denervated skeletal muscle result from the disuse caused by paralysis of the muscle, and from the loss of special myotrophic substances. Proteins extracted from rats' or sheep's sciatic nerves have been shown to ameliorate the atrophy of hindlimb muscles denervated for 7 days in adult rats or mice when administered by daily intramuscular or intraperitoneal injections respectively. The present investigation was undertaken to examine the effects of denervation and myotrophic effects of nerve extract on several physiological, biochemical and morphological parameters during the postnatal period of growth in mice.

The right hindlimbs of male C57BL mice were denervated at 2 weeks and redenervated at 4 weeks of age. During a 4-week period of denervation the mice either were untreated (UnRx) or received daily intraperitoneal injections of sheep nerve extract (500 mg protein/kg body weight/day) (Rx). Fast-twitch muscles from both denervated (DN) and contralateral normally innervated (NOR) hindlimbs were examined for one or more of the following parameters: wet weight, cross-sectional areas of fibers (CSA), content of total, soluble, myofibrillar and sarcoplasmic proteins, content of parvalbumin, twitch and tetanus tensions (Po, Pt), time to peak twitch tension (TTP), half-relaxation time (½RT) and post-tetanic potentiation (PTP). The muscles studied were extensor digitorum longus (EDL), tibialis anterior (TA) and gastrocnemius (G). Injections of extract had only a small effect on the atrophy of the denervated muscles. DN-Rx muscles exhibited 30% larger CSA of type II fibres (EDL) and 26% stronger tetanus tension (EDL) than DN-UnRx muscles. There was no significant effect on quantities or proportions of general muscle proteins (TA, G). However, injections of nerve extract prevented completely the decrease of parvalbumin (EDL, TA, G) and over 50% of the increases of TTP and ½RT (EDL) in denervated muscles. Thus the ameliorative effects of nerve extract on denervated muscles in young mice are greater on time- than strength-related changes in contractile function. The nerve extract also affected NOR muscles. The wet weights (TA, G) and content of sarcoplasmic protein (G) were smaller in NOR-Rx than NOR-UnRx muscles.

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335.5 MOTOR NERVE TERMINAL OUTGROWTH INDUCES RAPID MODIFICATION OF JUNCTIONAL ACETYLCHOLINE RECEPTOR CLUSTERS IN ADULT MAMMALIAN SKELETAL MUSCLE. W-C Yee* and A. Pestronk. Neuromuscular Division, Johns Hopkins Sch. of Med., Baltimore, MD 21205.

Outgrowth of motor nerve terminals at neuromuscular junctions (NMJs) occurs rapidly in response to denervation changes in muscle. Using a combined cholinesterase-silver stain to visualize NMJs, nerve terminal outgrowth (NTO) is manifest as an elongation of the NMJ (cholinesterase-staining area) with an increase in the number of nerve terminal branches within this area. In the present experiments we have begun to study whether NTO can induce changes in the stable cluster of acetylcholine receptors (AChRs) at the adult mammalian NMJ.

We first examined whether the NTO-induced increase in NMJ size results in elongation of the postsynaptic AChR cluster. NTO was evoked by using botulinum toxin to block neuromuscular transmission and cause denervation changes in the rat soleus muscle. Seven days later, a time when significant NMJ elongation could be demonstrated using the cholinesterase stain, junctional AChRs were visualized using an α-bungarotoxin-peroxidase-antiperoxidase technique. Our results showed that the length of the junctional AChR cluster increased from 40 ± 1 μm to 51 ± 1 μm over the 7 day period of NTO. This change was similar to the elongation in the cholinesterase staining area which increased from 39 ± 3 μm to 50 ± 2 μm over the same period. The similarity in the changes in cholinesterase staining and AChR clusters was also evident at the level of individual NMJs. Using a double staining technique, we found that the distributions of cholinesterase and AChRs were virtually identical at each normal or elongated NMJ. In contrast to these findings, no change in the length of junctional AChR clusters was seen after surgical denervation of muscle. This suggests that elongation of the AChR cluster is induced by NTO and is not a result of denervation changes in muscle per se.

We next studied whether the elongation of the junctional AChR cluster was accompanied by an increase in the number of AChRs at the NMJ. Using an $^{125}$I-α-bungarotoxin binding technique, we found that the number of junctional AChRs was significantly increased in soleus muscles with NMJ elongation. After NTO there were 5.5 ± 0.3 x $10^7$ AChRs/NMJ, a figure 62% higher than the 3.4 ± 0.4 x $10^7$ AChRs/NMJ found in controls.

Our data show that NTO results in elongation of the junctional AChR cluster and an increase in the number of AChRs within the cluster. Thus, NTO is capable of producing rapid and significant modifications in the previously stable postsynaptic AChR cluster at the mammalian NMJ.

335.6 COMPARISON OF THE EFFECTS OF DENERVATION AND NEUROMUSCULAR BLOCKADE ON ACETYLCHOLINE (ACh) RECEPTOR NUMBERS IN ADULT RAT SKELETAL MUSCLE. L. Bambrick* and T. Gordon (sponsored by Dr. S. Malhotra), Department of Pharmacology, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7.

The synthesis, incorporation and distribution of ACh receptors in skeletal muscle membranes is one of the most striking examples of muscle plasticity to neural influence and to levels of muscle activity. We have examined whether the increase in extrajunctional ACh receptor following denervation can be accounted for by loss of neuromuscular transmission. We have compared the binding of [$^{125}$I]-iodo-α-bungarotoxin [$^{125}$I-BTX] to muscle homogenates prepared from denervated or botulinum toxin (BOTX) poisoned triceps surae muscles (excluding soleus). As BOTX acts presynaptically to block ACh release this blockade provides a chemically silent but otherwise intact neuromuscular junction. Increased $^{125}$I-BTX binding was first observed after 3 days and the numbers rose exponentially ($\tau$=2.8 days) in denervated and BOTX blocked muscles, attaining maximum values, shown in the table for 7-8 muscles each, after 9 days.

| Triceps surae muscles | $^{125}$I-BTX sites (X±S.E.) | |
|---|---|---|
| | fmoles/mg muscle protein | fmoles/mg muscle weight |
| Denervated | 25.9±3 | 2.6±0.2 |
| BOTX treated | 24.5±3 | 2.9±0.5 |

It is clear that the effects of BOTX treatment and denervation were equivalent. This was also true for muscle atrophy as seen by a decrease of 28% in muscle weight with respect to untreated controls. These results, together with previous data (Berg and Hall, J. Physiol. 244: 659, 1975; Brown et al., J. Physiol. 327: 29, 1983; Lavoie et al., Exp. Neurol. 54: 148, 1977) are consistent with the idea that blockade of ACh receptors or of the release of ACh is equivalent to denervation in promoting skeletal muscle hypersensitivity. (Supported by the AHF and the AHFMR).

335.7 CHRONIC ELECTRICAL STIMULATION OF AXOTOMIZED NEURONS IN THE RABBIT. R. Orozco*, T. Gordon, L.A. Davis* and G. Goldsand*. Department of Pharmacology and Surgery, University of Alberta, Edmonton, Alberta, Canada, T6H 2H7.

When a mammalian peripheral nerve is severed, there is an initial decline, then stabilization in the diameter of the fibers which remain in continuity with the cell bodies (Davis et al. J. Physiol. 285: 543, 1978). This atrophy can be reversed if functional connections are remade with denervated end-organs, as a result of either re-establishing neural activity or by allowing the flow of trophic factors. Two observations, namely that the most rapid atrophy coincided with a fall in neuronal activity in the injured neurons, and that the silenced sensory axons atrophied relatively more than active motor axons (Hoffer et al. Brain Research, 178, 347-361, 1979) suggested to us that superimposing neural activity might reverse atrophy in axotomized neurones.

Extracellular cuff electrodes were bilaterally implanted around the sciatic nerve and the common peroneal or tibial nerve in 12 rabbit hindlimbs for chronic stimulation and monitoring of the compound action potential (CAP). Evoked CAP amplitude and latency were measured and used as a reference for nerve diameter. In a second operation common peroneal or tibial nerves were cut bilaterally and ligated distal to their cuff electrodes. Electrical stimulation of the proximal segment of the nerve was carried out at a frequency of 10 Hz, 8 hours a day for up to 200 days in one limb while the other served as control. CAP and latency were again recorded at 5 days intervals to monitor changes in axon diameter. Evoked CAP amplitude declined to about 25% of preoperative values with a similar time course irrespective of whether the axotomized neurons were chronically stimulated or not. An increase in CAP latency was also similar in stimulated and nonstimulated nerves.

The present study shows that chronic electrical stimulation neither prevents atrophy in axotomized neurons nor stimulates recovery of atrophic neurons. Thus, the most likely explanation for the recovery of axotomized neurons after reinnervation is the reinstitution of trophic factors.

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335.8 ACTIVITY-DEPENDENT REGULATION OF ACH SYNTHESIS IN CULTURED CILIARY GANGLION J.B. Tuttle and D.B. Gray, Department of Physiology and Neurobiology, University of Connecticut, Storrs, CT 06268.

Recent work from this laboratory has demonstrated that co-culture of striated muscle cells with ciliary ganglion neurons can not only increase basal acetylcholine (ACh) synthesis and choline acetyltransferase (ChAT) in neurons, but allow an acceleration of ACh synthesis following stimulation-induced transmitter release. This latter characteristic is considered to be a measure of synaptic maturity. In addition, muscle co-culture with E9 ciliary ganglion neurons removes the dependence of these neurons upon the presence of chick embryo extract for survival past 3 days in vitro.

In order to test if synaptic transmission or electrical activity is responsible for these effects, co-cultures were chronically exposed to tetrodotoxin (TTX) at concentrations of 1 nM to 1 uM which have been shown to completely block spike propagation in the ciliary ganglion. Even at 1 uM, there was no significant effect of TTX on survival in co-cultures, and less than a 10% decrease in survival of cultures of neurons alone.

In cultures of neurons alone, ACh synthesis rates in basal conditions and in stimulus-induced accelerations were not affected by chronic exposure to TTX. However, in muscle co-culture, 30-50% of the stimulus-induced acceleration of ACh synthesis was sensitive to TTX at concentrations of at least 1 nM. Acute exposure of neurons to TTX during synthesis measurements had no effect. These experiments were also repeated on cultures of neurons plated upon membrane fragments of lysed myotubes. This substrate also supports the development in vitro, of a compensatory ACh synthetic capacity following stimulation, although not as great as in co-culture with live muscle. Chronic exposure to TTX had no significant effect upon synthesis rates in these cultures.

Thus, TTX-sensitive electrical activity may be responsible for a portion of the compensatory increase in ACh synthesis trophically induced in ciliary ganglion neurons by co-culture with live muscle. Although it is tempting to conclude that this trophic interaction may be mediated by both activity dependent factors and membrane contact factors (as represented by membrane fragments), electrical activity not sensitive to TTX, such as calcium spikes, cannot be ruled out. However, chronic exposure of cultures to high K+ medium (28mM KCl) which depolarizes cells and might be expected to increase inward calcium currents, increases ACh synthesis rates overall, but lowers the amount of compensatory ACh synthesis following stimulation when expressed as a percentage of basal rates. Supported by the U.S. Army Res. Offices and BSN 8410581.

335.9 FOREBRAIN CHOLINERGIC NEURONS SURVIVE AND DIFFERENTIATE IN CULTURE IN THE ABSENCE OF ASTROCYTES. J. Hartikka* and F. Hefti. (SPON: A. Dravid). Dept. of Neurology, University of Miami School of Medicine, Miami, FL 33101.

Forebrain cholinergic neurons degenerate selectively in senile dementia. Knowing the factors which are needed for the development and survival of these neurons might help to find the reason for their degeneration. In vivo, neurons are surrounded by glia cells, astrocytes being a major population of them. Astrocytes seem to be important for maintenance of function of neurons in the mature brain. Furthermore, it has been proposed that they guide neuronal growth and promote differentiation during development. We have therefore studied the effects of astrocytes on the development of forebrain cholinergic neurons in primary cell cultures.

Cultures of dissociated cells were prepared from fetal (E17) rat septum. They were grown in a modified L-15 medium. After ten days in vitro the amount of protein and the activity of choline acetyltransferase (ChAT) were determined. The activity of ChAT, the specific enzyme marker for cholinergic neurons, provived an index for differentiation. Another set of cultures was taken for cell counting. Total number of cells was counted by staining the nuclei with bisbenzimide (Hoechst 33258). Astrocytes were identified by their content of glial fibrillary acidic protein which was visualized immunocytochemically. Cholinergic neurons were identified using ChAT immunocytochemistry and acetylcholinesterase cytochemistry. The proliferation of non-neuronal cells was suppressed by using two different compounds: cytosine arabinoside (Ara-C) and aphidicolin. Ara-C is a nucleotide analogue which terminates DNA replication. Aphidicolin is a specific inhibitor of DNA polymerase alpha, which is the enzyme needed for DNA replication in dividing eucaryotic cells. Using either of these compounds three types of cultures were prepared: 1) control cultures in which non-neuronal cells were allowed to proliferate freely. After ten days about 50% of the cells in these cultures were identified as astrocytes; 2) mixed cultures containing 5-10% astrocytes; 3) pure neuronal cultures containing less than 2.5% astrocytes.

The results showed that the survival of cholinergic neurons was independent of the presence of astrocytes. The ChAT activity per cholinergic neuron was significantly higher in, both, mixed cultures and pure neuronal cultures, than in control cultures. As compared to the control cultures ChAT activity was increased in mixed cultures and in pure neuronal cultures by 80% and by 130%, respectively.

The results suggest that forebrain cholinergic neurons survive and grow in culture for ten days in the absence of astrocytes. No beneficial effect from astrocytes on neuronal differentiation was observed. Astrocytes even seem to attenuate the expression of cholinergic properties in these cultures.

335.10 THE INFLUENCE OF TARGET AND NON-TARGET CELLS ON THE DEVELOPMENT OF SEPTAL CHOLINERGIC NEURONS: A HISTOCHEMICAL STUDY OF REAGGREGATING CELL CULTURES. J. Hsiang,* B.H. Wainer, A. Heller and P.C. Hoffmann (SPON: A. Levey). Dept. of Pharmacological and Physiological Sciences, University of Chicago, Chicago, IL 60637.

Cholinergic neurons of the septohippocampal pathway are known to have an important role in cognitive function. To better understand the factors responsible for the development and maintenance of this system, we have studied these cells in rotation-mediated reaggregating cell cultures. Brain cells were obtained by dissociation with 0.1% trypsin from the following areas of 15 day fetal mouse brain: (i) septum containing the cholinergic projection cells, (ii) hippocampus containing their target cells, (iii) and cerebellum containing non-target cells. The following combinations were aggregated in 10% serum supplemented medium in a rotatory incubator: (a) septal cells alone; (b) hippocampal cells alone; (c) cerebellar cells alone ($10 \times 10^6$ cells for each case), (d) septal-hippocampal cells, together and (e) septal-cerebellar cells, together ($5 \times 10^6$ cells from each area). After 6 days, the aggregates were transfered to serum-free defined medium for 2 weeks. Serial sections (60 $\mu$m) prepared from paraformaldehyde-fixed aggregates were studied using acetyl-cholinesterase (AChE) histochemistry. In all aggregates containing septal cells large AChE-positive neurons were present with prominent proximal dendrites. Sections from aggregates containing either hippocampal or cerebellar cells alone contained only a few weakly positive cells in which proximal processes were rarely seen. However, septohippocampal coaggregates displayed abundant fine-caliber varicose fibers suggestive of axonal arborizations. This fiber pattern was found much less often in aggregates of either septum alone, or septal-cerebellum and in many of these sections both coarse and fine fibers appeared to have degenerated. In aggregates with hippocampal and cerebellar cells alone, no fine varicose fibers were seen. AChE-positive cells with proximal dendrites (counted in randomly selected sections) were significantly more numerous in septal-hippocampal coaggregates (5104 cells) than in septal aggregates (1969 cells) or septal-cerebellar coaggregates (710) ($p < 0.001$ in both cases). These results, taken together, suggest that hippocampal target cells influence the development of cholinergic neurons. Supported by NS-17661, HD-04583, MH 28942, and the Brain Research Foundation (The University of Chicago).

335.11 CONTROL OF MYELINATION IN THE FISH OPTIC NERVE. R.Karam*, A. Maggs*and J. Scholes* MRC Cell Biophysics Unit, King's College London, 26-29 Drury Lane, London WC 2. UK.

The optic nerve in fish is comprehensively myelinated. The only exception is a discrete bundle of immature fibres along the ventral edge of the nerve. This persists throughout growth during adult life and usually contains around 25,000 unmyelinated axons. New fibres are continually added to this bundle, just as older ones become myelinated, at a rate that accumulates about 250,000 myelinated fibres in the optic nerve by 6 months age. The number of axons in the unmyelinated bundle thus reflects some balance achieved between the rate of new fibre production and the rate at which oligodendrocytes proliferate and myelinate them. It came as a surprise to find we could change this number by changing the growth rate of the fish.

Growth in 6 month-old Tilapia nilotica brood-mates was either slowed by crowding and restricted feeding, or accelerated by free-range conditions and more food, for a period of 1 month before sacrifice when the optic nerves were prepared for electron microscopy. Normal brood-mate controls were sacrificed at the beginning of the experiments. Compared with a rough estimate of around 250,000 myelinated fibres, representative unmyelinated fibre counts in these fish were: NORMAL, 25,000; SLOW, 15,000 and FAST, 45,000.

These figures show that some rate limiting step prevents myelination keeping up with new fibre production. The fibre diameter profile among the unmyelinated axons agrees with this interpretation: the increase in numbers with rapid growth comes from proliferation of the very smallest, uniformly sized (ca. 0.15$\mu$m)new axons, rather than from the minority of fibres that begin to increase their diameter (upto 0.25$\mu$m) just before they myelinate.

We wondered if the rate limiting step could be the time taken by new fibres to colonise the optic tectum and form synapses there. We investigated this idea using two approaches, (1) by removing the tectum to see if preventing or delaying synapse formation increases the number of unmyelinated fibres seen in the growing optic nerve and (2) by examining retrograde transport of labelled precursors and markers for signs of a correlation between myelination and synapse formation.

335.12 MYELIN BASIC PROTEIN EXPRESSION IS ENHANCED BY NEURONS. L. Bologa, Y. Aizenman*, and J. deVellis, Beckman Research Institute of the City of Hope, Duarte, CA 91010 and UCLA School of Medicine, Los Angeles, CA 90024.

Myelin basic protein (MBP) is a marker for differentiated oligodendrocytes. Though previous results suggested that the expression of MBP is enhanced by neurons, this has not been positively proven. We present now direct evidence that the expression of MBP by cultured oligodendrocytes is enhanced by neurons. Cell cultures from total brain of neonatal rats and pure neuronal cultures from embryonic rat brain were prepared. At 7 days in vitro, the total brain cell cultures were divided into 3 groups. Group (a) received pure neurons; group (b) received neuronal conditioned medium; group (c) served as control. Three days later the cultures were fixed and immunostained. Results showed that in groups (a) and (b) the expression of MBP was enhanced. This indicates that neurons and soluble factors released by them enhance the expression of MBP.

335.13 EXTRACELLULAR PROTEOLYSIS IN CNS HISTOGENESIS: EXPRESSION OF PLASMINOGEN ACTIVATOR ACTIVITY BY THE DIFFERENTIATING ASTROCYTES. Nurit Kalderon. The Rockefeller Univ., New York, NY 10021

The role of extracellular proteolysis as a modulator of the cytoarchitecture of the developing and regenerating nervous system is being studied; specifically, of the serum proteolytic system, the plasmin-generating system. This system is composed of plasminogen and plasminogen activator (PA), and their product is the protease plasmin. It has been established by studies performed in this laboratory that the plasmin-generating system is expressed as well as modulated at certain developmental stages by the differentiating nervous system. Results show that this system is active at those stages when glia proliferate: (A) in embryonic spinal cord both in vivo (1985, submitted) and in vitro (J.Neurosci.Res. 1982 8:509); and (B) in purified Schwann cell populations, where PA activity is expressed predominantly in the proliferating cell populations (PNAS 1984 81:7216).

The following study is focused on the identification of the glial cell types which produce PA during CNS histogenesis and the characterization of the mode of expression of PA activity as a function of cell differentiation. Purified astrocytes and oligodendrocytes in cell culture were obtained following the procedure of McCarthy & de Vellis (J. Cell Biol. 1980 85:890). These glial cells were dissociated from cerebral tissue of rat pups at age P2-P3 (postnatal days) and the two cell types were separated 8-9 days later, i.e., corresponding to the age of P10-P12. The extracellular and cellular PA activities were determined on the purified cell populations. Extracellular PA activity was detected in the growth medium of the astrocytes but not of the oligodendrocytes. Astrocytes expressed PA cellular activity, whereas no cellular PA activity was detected in the oligodendrocytes (P11 or P15) which seemed to exhibit some protease inhibitory activity. Astrocytic cellular PA specific activity was determined as a function of their age during the period corresponding to P11-P24. The highest specific activity, 0.2 Ploug U/mg protein, was monitored at P15; this activity gradually declined with age (P18, P22, P24) and vanished on day P24. These results correlate with reported data that PA specific activity in postnatal rat cerebrum declines as function of tissue development and levels off at the age of P25 (Brain Res. 1981 216:361).

Results so far suggest that the Schwann cell and the premature astrocyte are the "suppliers" of PA activity in the developing nervous tissues. Proteolytic activity was detected in regenerating injured sciatic nerve but not in the injured optic nerve (Acta Neuropathol. 1982 58:224). It is speculated that whereas the Schwann cell retains the capacity to express PA activity in regenerating PNS, the astrocyte upon maturation loses this capacity, hence, the deficiency in extracellular proteolysis in mature or injured CNS.

335.14 INTERCELLULAR TRANSFER OF HRP LABEL IN CRAYFISH GIANT AXONS. R.A. Sheller*, K.R. Seshan*, T.A. Viancour, and G.D. Bittner (SPON: M.A. Olivo) Zool., U. Texas Austin, TX 78712 and Biol. Sci., U. Maryland, Baltimore Cty, Catonsville, MD 21228.

Intercellular transfer of the protein markers HRP and wheat germ agglutin (WGA)-HRP has been observed among crayfish giant axons and glial cells. The crayfish ventral nerve cord has two lateral giant axons (LGAs) and two medial giant axons (MGAs). The LGA is a functional unit composed of a chain of electrically coupled homologous single lateral giant axons (SLGAs). Each SLGA arises from a cell body located in a caudal ganglion and extends rostrally to form electrotonic gap junctions with the next-most anterior SLGA. Each MGA arises from a cell body in the supraesophageal ganglion and extends the length of the nerve cord.

When HRP is intracellularly iontophoresed in vitro (physiological saline containing 13.5 mM Ca++) into an SLGA in a nerve cord ligated anterior to the first abdominal ganglion, HRP injected posterior to the second abdominal ganglion transfers to an adjacent SLGA about 50% of the time. HRP transfers to the caudal SLGA (30%) significantly more often ($p<.01$) than it transfers to the rostral SLGA (20%). When HRP injections were performed in a reduced Ca++ (1.5 mM) saline with added Ca++ antagonists (5mM Mn++ and 5mM Co++), then only one cell in 44 transferred in either direction.

It is difficult to detect HRP transfer from giant axons to adaxonal glia, if HRP is iontophoresed into a giant axon and then maintained at 20°C before developing with DAB. However if the axon is incubated at 30°C for 2 hours after injecting the HRP at 20°C, HRP is seen in adaxonal glia, in some cases obviously in vesicles. It is also difficult to see evidence of HRP uptake in giant axons if the HRP is placed extracellularly to the axon at 20°C. However WGA-HRP placed extracellularly at 20°C for 1-3 hours, is readily taken up into the axons, where it is clearly seen in vesicles.

These data show that crayfish giant axons transfer protein markers between adjacent SLGAs and between giant axons and glia, possibly by an active process involving endocytosis/exocytosis. Previous studies (Bittner, G.D., Comp. Biochem. Physiol. 68A. :299-306, 1981) have shown that severed MGAs appear to depend upon glial sheath cells for long term survival (90-300 days) whereas severed LGAs depend primarily upon adjacent SLGAs for long term survival (>700 days) of enucleated axonal processes. Since both SLGAs have many 250-500 Å vesicles where they are closely apposed and MGAs have many 250-500 Å vesicles facing glial cells, this intercellular transfer may be the basis for long term survival of these axons when the axons are severed from their cell bodies.
Supported by NIH grant NS19764-01 to GDB.

335.15 IS THE INCORPORATION OF EXOGENOUS GM1 INTO NEUROBLASTOMA MEMBRANES MEDIATED BY A MEMBRANE PROTEIN? K.C. Leskawa*, R.E. Erwin* and E.L. Hogan (SPON: N.L. Banik). The Medical University of South Carolina, Charleston, SC 29425

To better understand the mechanisms by which exogenous gangliosides promote the sprouting and extension of neurites from nerve cells, the association of exogenous ganglioside GM1 with membranes of neuroblastoma cells in culture was studied. Previously we have reported that the presence of calcium ion inhibits three forms of GM1-membrane association, including incorporation into the membrane lipid bilayer (Leskawa et al., Soc. Neurosci., 1984 Anaheim, CA). Since divalent cations are known to render gangliosides more hydrophobic in a biphasic partitioning system (Quarles and Folch-Pi, 1967, J. Neurochem., 12: 543), incorporation into the membrane lipid bilayer was expected to increase in the presence of Ca++, rather than decrease, as was found.

Neuro-2A cells were grown in culture and synchronized in the G1/GO phase of the cell cycle by serum deprivation for 48 hr. Cells were removed from the substratum and incubated in HEPES-buffered saline containing 3H-GM1 at 0.1 mM. GM1 incorporated into the plasma membrane was defined as the radioactivity remaining in a cell pellet after (1) washing the cells with a serum-containing medium, (2) trypsin treatment and (3) washing in buffered saline (see Facci et al., 1984, J. Neurochem., 42: 299). Cells in suspension were pretreated with EDTA before incubation with 3H-GM1. One group was 'reloaded' with Ca++, another with Ca++, Mg++ and Mn++, and a third washed with saline only. After incubation, incorporation was determined as described above and no significant differences between the three groups were observed. These results suggest that inhibition of GM1 incorporation is due to Ca++ interactions with ganglioside micelles or oligomers in solution, rather than with Ca++ sensitive sites on the membrane.

These data suggest that incorporation of exogenous GM1 cannot be explained by considerations of lipophilicity alone, but rather that incorporation may be mediated by a membrane protein. This view is supported by the following: treatment of N2A cells with purified trypsin or chymotrypsin prior to incubation with 3H-GM1 resulted in decreased incorporation (6.2 and 3.8% of control values), whereas pretreatment with collagenase, neuraminidase and hyaluronidase resulted in little change. A preliminary analysis of varying pH upon membrane incorporation is also supportive of incorporation being protein-mediated, since pH optima were found. If incorporation was solely due to hydrophobic forces, one would expect altering pH to have an effect only at the pKa of sialic acid (pH 2.6-2.75). Thus, these studies suggest membrane transfer protein(s) being involved in an initial GM1 recognition event, which may then bring the ganglioside in close proximity to the lipid bilayer for incorporation to occur.
Supported by the South Carolina State Appropriations for Research.

335.16 NON-NEURONAL CELLS MEDIATE VIP STIMULATION OF NEURONAL SURVIVAL DURING DEVELOPMENT. D.E. Brenneman, S.W. d'Autremont* and E.A. Neale. Lab. of Dev. Neurobiol., NICHD, NIH, Bethesda, Md 20205

The regulation of neuronal development by neuropeptides was explored with various cell cultures. Vasoactive intestinal peptide (VIP) has been shown to influence neuronal survival in dissociated spinal cord cultures. The purpose of the present study was to examine the cellular mechanism of such a trophic effect. Previous studies have shown that blockade of electrical activity with tetrodotoxin (TTX) decreased the survival of cultured spinal cord neurons during a critical period in development. This effect was dependent on removal of endogenous conditioning substances. Tetrodotoxin blocks the spontaneous release of VIP-like immunoreactivity in dissociated spinal cord cultures. Addition of 0.1nM VIP to these cultures prevented the TTX-mediated neuronal cell death and neuronal death which occurred naturally during this period in development (days 7-21). In the present study, we investigated if this trophic action of VIP was due to a direct effect on neurons or if VIP was acting indirectly as a releasing agent for trophic substances derived from non-neuronal cells.

Three types of cultures were prepared from dissociated spinal cord tissue: non-neuronal background (BG) cultures, grown in MEM with 10% fetal calf serum; neuronal plus background (N+BG) cultures, grown in defined medium supplemented with 5% horse serum; and neuronal (N) cultures, grown in defined medium without serum. Control N cultures contained fewer neurons and substantially fewer background (GFAP positive) cells than N+BG cultures. A comparison was made of N versus N+BG cultures after TTX and/or VIP treatment. After 5 days treatment with TTX, both types of cultures exhibited a 35% decrease from neuronal cell counts of controls. Addition of 0.1 nM VIP plus TTX to N+BG cultures prevented neuronal cell death; whereas the same treatment in N cultures did not prevent TTX-mediated decreases in neuronal survival. VIP treatment alone had no significant effect on either N or N+BG cultures.

To further test the hypothesis that non-neuronal cells mediate the survival effects, 0.1 nM VIP was added to BG cultures for 3 days and then the conditioned medium (CM) was collected and tested on N cultures, which were previously shown not to respond to VIP treatment alone. This conditioned medium from VIP-stimulated BG cultures prevented neuronal cell death when added to the medium of N cultures treated with TTX. CM comprised 20% of the volume of the test cultures. Conditioned medium from BG cultures which were not treated with VIP had no protective action on N cultures. These data suggest that VIP stimulates the release of a survival promoting substance from non-neuronal cells, which is then competed for by a subpopulation of neurons whose survival is affected by changes in electrical activity.

335.17 TEMPORAL SEQUENCE OF ANGIOGENESIS IN NEURAL TRANSPLANT MODELS. J.M. Krum and J.M. Rosenstein. Dept. of Anatomy, George Washington Univ. Sch. of Med., Wash. D.C. 20037.

The survival of a neural transplant is ultimately dependent on the formation of functional vascular connections between it and host CNS. The temporal sequence with which renewal of transplant blood circulation occurs is related to the type of neural tissue grafted, the graft site, trauma incurred during surgery, or the mechanism of transplant revascularization.

Our studies have focused on the time course of transplant revascularization, utilizing both adult superior cervical ganglia (SCG) and fetal (E17-20) cortex cerebri allografted in the fourth ventricle of perinatal Wistar rats. Tritiated thymidine was administered one hour prior to sacrifice at postoperative time periods ranging from 4 hours to 4 weeks. The tissue was processed for light microscopic autoradiography and labelling indices (L.I.) of host parenchymal and pial endothelial cells within a 150 um area of the transplant were determined for each postoperative time period. The L.I. of transplant endothelial cells was also calculated.

In the atraumatic intraventricular operative procedure, cerebellar and medullary pial vessels subjacent to the graft site are only rarely damaged. After 4 hours, SCG transplants were firmly attached to the pial surfaces, and their intrinsic vessels collapsed and barely discernable. Labelling of pial vessels was not observed until 16 hours, with the highest endothelial L.I. occurring between 20 and 24 hours postoperatively. At these times, most SCG vessels were patent but unlabelled, although static pooling of red blood cells was present in many vascular profiles. This suggests a reperfusion of the original SCG vessels had occurred via inosculation with host pial vessels.

Fetal cortical transplants contained relatively few patent vessels which had a low endothelial L.I. at 24 hours postoperatively. The pliant fetal tissue was insinuated around pial vessels, in which the highest endothelial L.I. occurs at 48-72 hours postoperatively. At this time vessel profiles and L.I. increased within the developing transplants. At one week, pial, but not transplant, endothelial L.I. was significantly decreased. It appears the primary mechanism for supplying blood to both autonomic and CNS transplants is inosculation between host pial and intrinsic transplant vessels.

(Supported by NS-17468).

335.18 THE PRESENCE OF A BLOOD VESSEL OR PERIPHERAL NERVE IMPLANT INDUCES TROPHIC RESPONSES IN LESIONED RAT RETINA. E. Thomas Chappell* and James E. Turner (SPON: T. Troost). Department of Anatomy, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27103.

Unlike the embryonic vertebrate retina and the adult retina of many lower vertebrate species, the adult mammalian retina cannot regenerate at the histological level. However, under appropriate lesion conditions the adult retinal ganglion cell axons exhibit some capacity for collateral and/or regenerative sprouting. Moreover, it is clear that trophic substances are instrumental in the development, maintenance, and regeneration of neuronal populations. Presented here is evidence that the presence of a portion of mesenteric artery or sciatic nerve can affect the survival of retinal cell populations and contribute to the closure of a retinal incision. This evidence is obtained in a new in vivo model involving an incision in the dorsal surface of the eye in Sprague-Dawley rats, implantation of an arterial graft or peripheral nerve segment, and closure by microsuture. The histologic and morphometric data show the rescue of retinal cells (which normally die in the lesion area), close apposition of the wound margins (while there is a "die back" zone in the absence of an implant), and maintenance of retinal layer organization with a thickening of some layers (where disorganization is usually the case in lesions without implants). A double lesion paradigm indicates that the peripheral nerve implant can exert its influence over a distance of at least 1 mm. These results implicate the potential for trophic induction of plasticity in the adult mammalian retina. Supported by grant EY 04377 from the National Eye Institute awarded to James E. Turner.

## OPIATES, ENDORPHINS AND ENKEPHALINS: ANATOMICAL LOCALIZATION

336.1 SEPTAL LESIONS CAUSE A REVERSIBLE INCREASE IN ENKEPHALIN-LIKE BUT NOT DYNORPHIN A-LIKE IMMUNOREACTIVITY IN THE HIPPOCAMPUS. J.F. McGinty, J-S Hong, M. Blockus* and C.J. Frederickson*. Dept. Anatomy, East Carolina Univ. Sch. Med. Greenville, NC 27834, Lab Behav. Neurol. Toxicol. NIEHS/NIH Research Triangle Park, NC 27709 and Dept. Psychology, Univ. Texas at Dallas, Richardson, TX 75080.

Hippocampal dentate granule cells receive afferent input through the fornix from medial septal-diagnonal band (MS-DBB) nuclei and through the perforant pathway from entorhinal cortex. The MS-DBB projection is largely cholinergic whereas the lateral perforant and dentate granule-mossy fiber pathways contain proenkephalin-A and prodynorphin derived peptides, respectively. Four days after a unilateral transection of the fornix, the met-enkephalin content of the ipsilateral hippocampus was demonstrated to be significantly increased (Hong and Schmid, Brain Res. 205:415, 1981). In this study, we deafferented the hippocampus of its MS-DBB projection and examined enkephalin and dynorphin A immunoreactivity in the hippocampal formation at 3, 10, 16, 21, and 30 days post lesion. Bilateral electrolytic lesions (1mA for 30 sec) of the MS-DBB area were performed on male Sprague-Dawley rats. On the appropriate post lesion day, lesioned and control rats were perfused with formaldehyde for leu-enkephalin and dynorphin A (1-17) immunostaining of adjacent sections through the hippocampal formation and entorhinal cortex. MS-DBB lesions, verified by Nissl stained sections through the extent of the electrode site, caused a transient increase in enkephalin immunostaining in the lateral perforant pathway which was especially prominent in the outer molecular layer of the ventral dentate fascia. This increase in enkephalin immunostaining was maximal at 3 and 10 days and was not reliably different from control brains by day 21 post lesion. Dynorphin immunostaining in the dentate granule cell-mossy fiber pathway of lesioned rats did not appear to differ from control rats at any time point examined. Radioimmunoassays for enkephalin and dynorphin on similarly treated rats at the same time points are underway. The time course of the observed changes in perforant path enkephalin immunostaining parallels the transient increase in zinc dithizonate density in mossy fibers (Stewart, G. et al., Brain Research 290:43, 1984) as well as the time course of sympathetic fiber ingrowth (Loy and Moore, Exper. Neurol. 57:645, 1977) into the hippocampus after septal lesions.

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336.2 ENKEPHALIN FIBERS IN SYMPATHETIC NUCLEAR REGIONS: SEGMENTAL VS CENTRAL ORIGIN. M.A. Romagnano and R.W. Hamill. Dept of Neurology, Monroe Community Hosp. U of Roch Med Ctr., Rochester, NY 14603

Previous studies have demonstrated a ladder-like network of enkephalin (Enk) fibers in thoracolumbar spinal cord coinciding with the distribution of preganglionic sympathetic nuclear regions. The present studies in the rat employ transections, hemisections, dorsal and/or ventral rhizotomies to determine whether Enk fibers in sympathetic nuclei are of central or intraspinal origin.

Spinal cord transections and hemisections are performed at T5/6 or C8-T1. Dorsal and/or ventral roots are severed at levels T5-T10 or C7-T5. Animals are perfused with Zamboni's fixative one week or one month following surgery. Spinal cords are removed and 40µ horizontal serial vibratome sections are stained for Enk by the unlabeled antibody method of immunocytochemistry. Sections are incubated for 48-72 hrs at 4°C in the primary antiserum used at a dilution of 1/500-1/2000 (D.S.Sundberg).

Enk immunoreactivity is not altered in those segments with severed dorsal and/or ventral roots when compared to control sections. Likewise, spinal cord hemisections at T5/6 or C8-T1 resulted in no apparent changes in the distribution of Enk on the ipsilateral or contralateral side below the lesion.

One week following C8-T1 spinal transection there is a large decrease in Enk fibers in the intermediolateralis nucleus,pars principalis (ILp), intermediolateralis nucleus,pars funicularis (ILf) and dorsal commissural nucleus. Moderate decreases in Enk immunoreactivity is found in the intercalatus spinalis nucleus (IC) and the intercalatus nucleus, pars paraependylmalis (ICpe). One month following the C8-T1 spinal transection there is a greater decrease in Enk immunoreactivity in the ILp, ILf and dorsal commissural nuclei when compared to one week survival times. The ILp cell clusters appear completely devoid of immunoreactivity while a moderate decrease in immunoreactivity is still evident in the IC and ICpe nuclei compared to control sections.

The resulting changes in the distribution of Enk in the sympathetic nuclei is similar at both one week and one month following T5/6 spinal transection. Below the lesion there is a moderate decrease in Enk staining in ILp, ILf and dorsal commissural nuclei while there is a moderate-slight loss of fibers seen in the IC and ICpe nuclei. Above the lesion the Enk distribution pattern appears similar to that found in control sections.

These results indicate that: 1. Enk fibers found in thoracolumbar sympathetic nuclei are of both central and intraspinal origin; 2. descending (suprasegmental) central Enk pathways primarily project to the ILp, ILf and dorsal commissural nuclei; 3. intraspinal (segmental) Enk pathways appear to primarily exist in IC and ICpe nuclear areas. These new observations suggest that specific patterns exist for supraspinal and intraspinal Enk pathways.

336.3 CO-DISTRIBUTION OF DYNORPHIN B AND OXYTOCIN IN CERTAIN HYPOTHALAMIC NEURONS IN THE RAT. B. Quinn and E. Weber, Institute for Advanced Biomedical Research, Oregon Health Sciences University, Portland, OR 97201.

Immunohistochemical studies from a number of laboratories have demonstrated that several hypothalamic hormones (including oxytocin, vasopressin, and CRF) may be organized into different neuronal subpopulations, in which different opioid neuropeptides are expressed in each subpopulation. The differential co-storage of these neuropeptides may be important in determining the roles of different components of the hypothalamo-pituitary axis.

This study was undertaken to examine the distribution of peptides derived from the pro-dynorphin precursor throughout the magnocellular system. Male rats were treated with 50 ug of colchicine icv 48 hours prior to perfusion with buffered 4% paraformaldehyde/5% sucrose, and the brains were immersed overnight in 12% sucrose/2% polyethylene glycol (MW 400). The tissue was then frozen in dichlorodifluromethane in a liquid nitrogen bath. Serial 2-micron frozen sections were stained with antisera directed against oxytocin, vasopressin, met-enkephalin-arg-gly-leu, and several pro-dynorphin products: dynorphin A(1-17), dynorphin A(1-8), dynorphin B, and alpha-neo endorphin. Immunoreactivity was visualized using fluorescein-labeled second antibodies.

In the rostral hypothalamus, in the nucleus of the anterior commisure, all or nearly all oxytocin perikarya were also immunoreactive, in adjacent sections, for dynorphin B. These cells also showed some staining for alpha-neo-endorphin, but not for dynorphin A or dynorphin(1-8), or met-enkephalin-arg-gly-leu. In contrast, moving caudally, in the paraventricular and supraoptic nuclei very few oxytocin cells showed dynorphin B immunoreactivity. However, in these areas most of the vasopressin perikarya stained with each pro-dynorphin antiserum.

These results demonstrate the first immunohistochemical evidence for the same opioid peptide colocalized in both the oxytocin and vasopressin systems. Further, they suggest a potential neuropharmacological distinction in the release of oxytocin by rostral vs. caudal hypothalamic neurons, which could support potentially distinct physiological roles for these different oxytocinergic groups.

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336.4 IMMUNOCYTOCHEMISTRY OF LEUCINE-ENKEPHALIN AND DYNORPHIN B IN SPINAL CORD AND DORSAL ROOT GANGLION NEURONS GROWN IN CULTURE. D. Durand*, M.A. Werz and R.L. Macdonald, (SPON: L.T. Rutledge), Dept. of Neurology, Univ. of Michigan, Ann Arbor, MI, 48109.

Opioid receptors in the spinal cord are localized at least in part on primary afferent fibers. In addition, opioid receptors are present also on dorsal root ganglion (DRG) neuron somatic membranes. We have investigated the actions of opioids using intracellular techniques and have determined that mu-, delta-, and kappa- receptors are present on the somata of mouse DRG neurons grown in primary dissociated cell culture where binding of opioids to receptors results in a decrease of calcium influx (Werz and Macdonald, JPET, 227:394-402, 1983). It is likely that opioid receptors on primary afferent terminals are coupled to similar ion channels since opioids decrease neurotransmitter release from such fibers (Macdonald & Nelson, Science, 199:1449-51, 1978). It would be of interest to observe decreases of calcium influx following release of opioids from neurons. As a prelude to such investigation, we have studied the distribution of opioid peptides within primary dissociated cell cultures of spinal cord and dorsal root ganglia using the peroxidase-antiperoxidase technique of Sternberger. Two antisera have been emloyed, one directed against dynorphin B and one directed against leucine-enkephalin, to allow differentiation of the two opioid systems within spinal cord. Specificity of the immunohistochemical reactions was tested by carrying out primary incubations in the presence of a saturating concentration of the respective synthetic peptide and by crossblocking experiments distinguishing leucine-enkephalin immunoreactivity from dynorphin B immunoreactivity.

We observed small (<20 μm), multipolar spinal cord neurons that were immunoreactive for leucine-enkephalin. Spinal cord neurons positive for dynorphin B were not observed. In contrast, a small number of large DRG neuron somata were positively stained for dynorphin B but not for leucine-enkephalin. The differential localization of leucine-enkephalin and dynorphin B suggests different roles for these opioid systems in processing information within the spinal cord. Since opioid receptors are present on a subpopulation of DRG neuron somata, it would be of interest to determine if these same neurons are immunoreactive for dynorphin. Such a finding might suggest that opioid receptors on DRG neurons are autoreceptors.

We thank Dr. Stanley J. Watson for his kind gift of antisera. Supported by grant BNS-8118762.

336.5 PRO-DYNORPHIN IN THE ANTERIOR LOBE: ANATOMICAL, BIOCHEMICAL AND MOLECULAR BIOLOGICAL STUDIES. H. Khachaturian, T.G. Sherman, R.M. Dores, S.J. Watson and H. Akil. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109, U.S.A.

The existence of dynorphin in the pituitary anterior lobe (AL) has been previously described (Seizinger et al., BBRC, 103: 256-263, 1981). However, little is known about the anatomical or biosynthetic origin of this opioid in AL. What is established is that the dynorphin-like immunoreactivity in AL tends to be of higher molecular weight forms. Others (Civelli, Douglass and Herbert, personal communication), however, have had difficulty detecting pro-dynorphin mRNA in AL, bringing into question the anatomical origin of AL dynorphin. This problem is also underscored by the difficulty in visualizing pro-dynorphin peptides in AL using classical immunohistochemical tools. We now report our efforts in further characterizing this opioid family in AL.

Dynorphin immunoreactive cells can be visualized in the AL of colchicine-treated male Sprague-Dawley rats following chronic footshock stress. In these animals, both dynorphin A and dynorphin B immunoreactivities are present in a subpopulation of AL cells, which are distinct from the POMC containing corticotrophs. Dynorphin-like material can also be visualized in some AL cells in developing rats beginning at E17. To further characterize the precursor and its product, we have studied pro-dynorphin mRNA using a rat genomic probe from Civelli et al. (PNAS, in press, 1985). Anterior lobe dynorphin mRNA exhibits an apparent MW similar to that in hypothalamus and striatum (2400 nucleotides). Finally, we have been successful in showing pro-dynorphin biosynthesis in AL short-term cell cultures using a classical pulse-labelling approach with [$^3$H]leucine. Three immunoaffinity columns were used for the purification: anti-dynorphin A (1-17), anti dynorphin A (1-8) and anti-pro-dynorphin Bridge Peptide. All 3 columns captured a common and unique 24K molecular species. Taken together, our results show the existence of pro-dynorphin mRNA and the translation of that message in AL. The system appears to become induced by repeated stress since dynorphin concentrations increase to an immunohistochemically detectable level. The exact type of AL cells which express the pro-dynorphin gene is currently under investigation.

This work was supported by NIMH Grant # MH39717 (SJW).

336.6 DYNORPHIN CELLS IN THE LATERAL HYPOTHALAMUS INNERVATE THE AMYGDALA, CENTRAL GRAY, PARABRACHIAL NUCLEUS AND DORSAL VAGAL COMPLEX. A.M. Zardetto-Smith, M. Moga, D. Magnuson, S. J. Watson and T.S. Gray, Dept. Anat., Loyola Stritch School of Medicine, Maywood, IL 60153 and Mental Health Res. Inst., Univ. Mich., Ann Arbor, MI 48109.

Previous studies have localized dynorphin(DYN)-immunoreactivity within the paraventricular, supraoptic, arcuate and lateral hypothalamic nuclei. The median eminence has been demonstrated to be a major recipient of projections from the magnocellular DYN-immunoreactive neurons. In the present study, evidence is presented that DYN-immunoreactive neurons in the lateral hypothalamus have extensive extrahypothalmic projections.

Male Long-Evans rats (150-250 gm) were anesthetized and unilateral 50-100 nl injections of the retrograde tracer fast blue were stereotaxically placed into the central nucleus of the amygdala (CNA), central gray (CG), parabrachial nucleus (PBN) or dorsal vagal complex (DVC). Intracerebroventricular injections of colchicine were administered 24-72 h prior to sacrifice. Postoperative survival after fast blue injections ranged from 7-12 days. Animals were perfused transcardially with 4% paraformaldehyde and brains were cut at 20 um using a vibratome. Sections were incubated overnight with primary antibody generated against dynorphin A or an adrenal enkephalin precursor fragment, BAM-22-P. The sections were then rinsed and incubated in a rhodamine-conjugated goat anti-rabbit immunogammaglobulin. After mounting, sections were examined for cells that contained both fast blue and rhodamine immunofluorescence.

DYN-immunoreactive retrogradely labeled neurons were observed in the posterolateral hypothalamus and perifornical area after injections into the CNA, CG, PBN and DVC. Occasionally retrogradely labeled DYN-immunoreactive neurons were seen in the paraventricular nucleus. In contrast, cells labeled for both fast blue and BAM-22-P were rarely observed.

The present study provides evidence for lateral hypothalamic dynorphinergic innervation of a number of brain nuclei that are thought to control autonomic outflow. This projection parallels, but is separate from, the pro-opiomelanocortin projections originating from the medial basal hypothalamus and arcuate nucleus. In addition, evidence was lacking for a strong, similarly projecting enkephalinergic pathway. A possible function of this DYN peptidergic projection may be to mediate the integration of autonomic responses that occurs during emotional and/or stress related behaviors. (Supported by NIH grant NS 20041).

336.7 **COMPARATIVE IMMUNOCYTOCHEMICAL DEMONSTRATION OF PEPTIDE F-, MET$^5$-ENKEPHALIN- AND MET$^5$-ENKEPHALIN-ARG$^6$-GLY$^7$-LEU$^8$-CONTAINING NEURONS IN THE RAT GASTROINTESTINAL TRACT.** Y.N. Wang, A.C. Church and R.J. Wyatt. Neuropsychiatry Branch, NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032.

Peptide F, a thirty-four amino acid fragment derived from the proenkephalin A molecule has been found in the brain and adrenal medulla of several species by radioimmunoassay. In the present study we report that Peptide F is present in the neuronal elements of the rat gastrointestinal (GI) tract and that the distribution appears to be similar to that of the proenkephalin derivatives, Met$^5$-enkephalin (Met-enk) and Met$^5$-enkephalin-Arg$^6$-Gly$^7$-Leu$^8$ (Met-enk-Arg-Gly-Leu). Esophagus, stomach, duodenum, jejunum, ileum and colon were fixed in periodate-lysine-paraformaldehyde (PLP) and sectioned with a cryostat (8-10 $\mu$m). Tissue sections were incubated first in antisera to either Peptide F, Met-enk or Met-enk-Arg-Gly-Leu, and then in fluorescein conjugated goat anti-rabbit IgG. Specificity of the Peptide F antiserum was established by incubating control tissues (adjacent slices) in antiserum previously absorbed with 10 $\mu$M synthetic Peptide F. Peptide F-containing neurons were found in all parts of the GI tract while the greatest number of immunoreactive neurons was observed in the duodenum. Peptide F-containing nerve cell bodies were mainly located in the myenteric plexus. Peptide F-containing nerve fibers were primarily present in the myenteric plexus, and the circular muscle layer while a few immunoreactive fibers were seen in the longitudinal muscle and the muscularis mucosa layers, the submucous plexus, and the mucosa. By comparing the distributions between Peptide F and the other related enkephalins we found that these three peptides have a similar distribution in the GI tract, and that they are most likely located in the same neurons. The results suggest that Peptide F, an intermediate of proenkephalin A, may play a physiological role within certain functional neurons of the enteric nervous system.

336.8 AUTORADIOGRAPHIC DIFFERENTIATION OF MULTIPLE OPIOID RECEPTOR SUBTYPES WITH SELECTIVE LIGANDS. Alfred Mansour, Michael E. Lewis, Henry Khachaturian, and Stanley J. Watson. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109, U.S.A.

Previous opioid receptor autoradiographic studies used ligands which are less selective than those available now. The present experiments were therefore designed to characterize the opioid receptor subtypes in the rat forebrain using [$^3$H]DAGO (Tyr-D-Ala-Gly-NMe-Phe-Gly-ol), a selective mu ligand, [$^3$H]DPDPE (D-Pen$^2$, D-Pen$^5$-enkephalin), a selective delta ligand, and [$^3$H]bremazocine, a kappa opiate (in the presence of 100 nM DAGO and DPDPE). Thaw-mounted coronal sections were incubated with either 4.85 nM DAGO, 36 nM DPDPE, or 0.89 nM bremazocine in 50 mM Tris HCl (pH 7.4) for 60 min at 25°C. These concentrations were determined to be 3 times the $K_d$ value of each of the ligands and therefore represented 76-78% receptor occupancy. Incubations were terminated by four consecutive Tris washes (30 sec for DAGO and DPDPE, 4 min for bremazocine at 4°C), followed by a 2 sec water (4°C) rinse. The sections were dried, apposed to tritium-sensitive LKB Ultrofilm with 14 step tritium standards (American Radiolabeled Chemicals, 0.0-489.1 uCi/g), stored at 25°C, developed, and analyzed densitometrically. In agreement with previous studies, the mu ligand labelled sites in a patchy distribution in the caudate-putamen, while the delta ligand labelled sites diffusely. Unlike previous reports, the striatal distribution of delta sites was not uniformly diffuse, but exhibited an increased density in cytoarchitecturally distinct subareas. The mu-delta differentiation was also evident in substantia nigra, where [$^3$H]DAGO and [$^3$H]DPDPE binding sites were concentrated in the pars compacta and pars reticulata, respectively. These findings illustrate the importance of using selective ligands to facilitate the autoradiographic discrimination of opioid receptor subtypes. [$^3$H]Bremazocine autoradiograms are not presently available, but will be presented at the meeting.

This work was supported by NIDA Grant #DA02265 and NIMH Grant # MH39717.

336.9 ALFENTANIL-INDUCED RIGIDITY IN THE RAT: THE ROLE OF THE NUCLEUS RAPHE PONTIS AND THE CAUDATE NUCLEUS. T.A. Blasco*, D. Lee, M. Amalric, N.R. Swerdlow, N. Ty Smith*, and G.F. Koob. Dept. of Basic and Clinical Research, Div. of Preclin. Neuroscience and Endocrinology, Scripps Clinic and Research Foundation, La Jolla, CA., Dept. of Anesthesiology, Univ. of Cal., San Diego.* (SPON: Rita Valentino)

Previous attempts to eliminate the rigidity associated with high-dose narcotic anesthesia in the operating room have proven only partially successful. Past laboratory work investigating the neuroanatomical sites of this rigidity have implicated both the nucleus raphe pontis (NRP) within the reticular formation (Broekkamp et al., Neurosci. Letters, 50:313-318, 1984) and the caudate nucleus (CN) within the basal ganglia (Havemann et al., Arc. of Pharmacol., 313:139-144, 1980). The present study, using systematically administered alfentanil (ALF), a new powerful, short-acting narcotic, and intracerebrally applied methylnaloxonium (MN), attempted to further elucidate the functional role of the NRP and the CN in rigidity.

Wistar rats were used in developing a dependable model of ALF-induced rigidity (ALF 0.5 mg/kg s.c.) as documented by a gastrocnemius electromyogram (EMG). The duration of this rigidity lasted approximately 30 minutes. The general ability of MN to antagonize rigidity was studied with intraventricular injections 15 minutes prior to the ALF treatment. The 2.0 ug and 4.0 ug doses prevented rigidity ($P < 0.005$), while a 0.5 ug dose demonstrated borderline antagonism ($P < 0.10$). In the NRP 0.125 ug and 0.5 ug of MN were able to significantly antagonize rigidity ($P < 0.005$) compared with a saline control. In the CN neither 1.0 ug or 4.0 ug antagonized rigidity. These observations demonstrate the NRP to be at least 10 times more sensitive to antagonism of rigidity by MN than the ventricular injections. The NRP was at least 32 times more sensitive to antagonism of rigidity by MN than the CN. Results suggest that the NRP may be an important site for the muscular rigidity associated with high dose narcotic administration. This work was supported in part by Janssen Pharmaceutical Company.

336.10 QUANTITATION OF LOCAL CEREBRAL GLUCOSE UTILIZATION DURING OPIOID DEPENDENCE AND NALOXONE-PRECIPITATED WITHDRAWAL. A.S. Kimes, R.C. Walovitch and E.D. London. Neuropharmacology Lab., NIDA Addiction Research Center, Baltimore, MD 21224.

Localization of the cerebral areas which may be activated or inhibited during opioid dependence and naloxone-precipitated morphine withdrawal have been studied using 2-deoxy-D-[1-$^{14}$C]glucose ([$^{14}$C]DG) mapping (Wooten, G. F. et al., Proc. Natl. Acad. Sci., 79:3360, 1982; Geary, W.A. and Wooten, G.F., Brain Res., 275:117, 1983). We have extended this work by measuring local cerebral glucose utilization (LCGU) in morphine-dependent or naloxone-precipitated abstinent rats. LCGU of both groups was compared to values in drug-naive controls. LCGU was measured by quantitative autoradiography using the [$^{14}$C]DG method of Sokoloff, L. et al. (J. Neurochem., 28:897, 1977). Fischer-344 male rats were made morphine-dependent by implantation of 75 mg morphine pellets (one on day 1 and 2 on day 4). Control rats were implanted with placebo pellets according to the same schedule. On day 8, each rat was prepared for the [$^{14}$C]DG procedure by implantation of femoral venous and arterial catheters, and was partially restrained by a lower body plaster cast. Injections of naloxone (0.5 mg/kg, s.c.) or saline were given 2 min before [$^{14}$C]DG (125 $\mu$ Ci/kg, i.v.). Naloxone produced prominent signs of withdrawal which included vocalization, teeth chattering, hyperactivity and nosebleeds. Concentrations of [$^{14}$C]DG and glucose were measured in 14 timed blood samples which were taken during the experimental period. The rats were overdosed with Na pentobarbital 45 min after the [$^{14}$C]DG injection. The brains were removed and frozen for cryostatic sectioning.

Although LCGU was relatively unchanged in morphine-dependent rats, there were significant increases in naloxone-precipitated abstinent rats. Many of the affected areas are rich in opioid receptors and/or have been related electrophysiologically to the withdrawal syndrome (Geary, W.A. and Wooten, G. F., J. Pharmacol. Exp. Therap., 225:234, 1983; Aghajanian, G., Nature, 276:186, 1978). These areas include the central gray, locus ceruleus, central and medial amygdaloid nuclei and anteroventral thalamic nucleus. In contrast to findings by Wooten et al., (Proc. Natl. Acad. Sci., 79:3360, 1982), increases in LCGU in the hippocampus during morphine dependence were not detected in our study. Possible reasons for this discrepancy include the following: 1) Using grey matter/white matter optical density ratios may have overestimated relative hippocampal LCGU in the morphine-treated rats studied by Wooten, G.F., et al.; 2) The morphine pellets were not removed from our rats prior to LCGU measurement, but Wooten, F. et al. did remove the pellets 2 hr before [$^{14}$C]DG injection; 3) Wooten, G. F., et al. used naloxone-injected morphine naive rats as controls.

337.1 INCREASED GROWTH OF MYELINATED PERIPHERAL NERVE INTO AGANGLIONIC SEGMENTS OF THE LS/LS MUTANT MOUSE. V.M. Tennyson, T.D. Pham*, T.P. Rothman, and M.D. Gershon. Dept. of Anatomy and Cell Biology, Columbia University, College of P & S, New York, N.Y., 10032.

The enteric nervous system is colonized by cells that migrate to the gut from the neural crest. In the ls/ls mutant mouse, the terminal hindgut fails to be colonized by neural crest-derived precursors of intrinsic neurons; thus, the terminal segment lacks myenteric and submucosal plexuses and the mouse develops congenital megacolon. We have postulated that the defect occurs because the microenvironment of the terminal hindgut is segmentally abnormal and does not permit migration and/or survival of the enteric neuronal precursors in the affected zone. There are nerve fibers in the aganglionic region. These fibers are supported by Schwann cells that express the high intensity of glial fibrillary acid protein (GFAP) that is normally characteristic of enteric glia. In order to determine whether neural supporting cells in the aganglionic ls/ls bowel are Schwann cells or enteric glia we have examined the ultrastructure of the nerves in the abnormal tissue in detail. We now report that in the absence of the intrinsic enteric innervation, the microenvironment of the gut permits or induces the ingrowth into both the presumptive myenteric region and the submucosa of large peripheral nerve bundles. We found that these bundles even contain myelinated axons. In a few cases, we observed neurons on the external surface of the aganglionic bowel within peripheral nerve bundles in regions where the external longitudinal muscle was absent. The supporting cells of the unmyelinated and myelinated nerves in the aganglionic ls/ls bowel all had the typical morphology of peripheral Schwann cells (individual ensheathment of axons, a basal lamina, no astrocytic contour). Large bundles of peripheral nerve containing neurons and myelinated axons were absent in the enteric plexuses of the terminal bowel of control mice, but they were found in the adventitia 50 to 100 um external to the longitudinal muscle. We suggest that the extracellular matrix of the terminal bowel of the control ls/ls mouse may prevent the ingrowth of the normal precursors of the glia as well as neurons of the enteric nervous system, but permit or encourage the ingrowth of abnormal numbers of extrinsic axons. These nerves may carry supporting cells into the gut where they express high levels of GFAP but are otherwise typical Schwann cells. Supported by Grants HD 17736, NS 15547, NS 11766, BNS 83-04904, and MOD 1-747

337.2 DEVELOPMENTAL POTENTIAL OF INNERVATED EMBRYONIC QUAIL GUT BACK TRANSPLANTED TO A NEURAL CREST MIGRATION PATHWAY OF CHICK EMBRYOS: INTERACTION WITH THE HOST'S CNS. T.P. Rothman, N.M. LeDouarin* and M.D. Gershon. Dept. Anat. & Cell Biol., Columbia Univ. P&S, NY and l'Inst. d'Embryol., CNRS, Nogent-sur-Marne, France.

The enteric nervous system (ENS) has a structure that resembles the brain and is different from that of other regions of the PNS. The neural crest-derived precursor cells that colonize the bowel and form the ENS proliferate extensively even after definitive enteric neurons can be recognized. It has been shown that precursor cells that co-exist with neurons in other developing peripheral ganglia will travel along neural crest migration pathways when grafted (back-transplanted) into younger embryos. The destinations and fate of the migrating cells reflect the extent of their determination at the time of grafting. In order to gain insight into the developmental capability of the primordial ENS and the enteric mesenchyme with which it interacts, embryonic quail duodenum (4 day) was grafted into neural crest migration pathways of chick embryos through which precursor cells normally migrate to the sympathetic ganglia and adrenal medulla (somites 20-21 of embryos of 20-24 somites). Grafts were placed between the somite and the neural tube and the embryos were killed at stage 35 (9 days). Donor cells in the chimeric embryos displayed the quail nuclear marker. Cells migrated from the gut into the dermis and dorsal mesenchyme of the host entering forming muscle and feather germs; however, no enteric cells participated in the formation of cartilage. In fact, the development of vertebrae was impeded unilaterally at the site of the graft. Quail cells also invaded the proximate dorsal root and sympathetic ganglia, the meninges, peripheral nerves, dorsal and ventral roots, and the adrenal medulla but not the gut. Moreover, there was migration of donor cells into the spinal cord where they entered the gray matter of the anterior horn. The host CNS reacted to the grafts. Large bundles of nerve fibers connected the spinal cord and the adjoining donor tissue. These fibers mixed with and became enveloped by whirls of elongate quail cells. There was also a unilateral hypertrophy of the spinal cord. These results are consistent with the hypothesis that the primordial ENS contains precursor cells that can leave the bowel and follow neural crest migratory pathways. Unlike other grafts involving peripheral ganglia, however, the back-transplantated bowel interacts with the developing CNS. This interaction, and especially the apparent local stimulation of host CNS growth by the grafts of bowel suggest that the CNS may respond to growth factors produced by the enteric mesenchyme. Supported by grants BNS-8204904, INT8413816, and NS15547.

337.3 POSTNATAL GENERATION OF NEURONS IN THE DEVELOPING ENTERIC NERVOUS SYSTEM. S. Benjamin*, T.P. Rothman and M.D. Gershon. Dept. Anatomy and Cell Biol., Columbia Univ. P&S, New York, NY.

During ontogeny of the mammalian enteric nervous system (ENS) peptide-containing neurons appear later than those containing small molecule neurotransmitters. It is possible that late-appearing neurons may be derived from a reservoir of proliferating neuroblasts that co-exists with neurons in the immature ENS. Experiments were thus done to determine for how long proliferating neural precursors persist following the first appearance of neurons in the developing bowel. $^{3}$H-Thymidine ($^{3}$H-TdR; 5 uc/g) was injected (i.p.) at 1 day, 7 days, 2 weeks and 29 days of postnatal life. For each developmental age, groups of animals were divided into several subsets. One group of animals received only 1 injection of the isotope. These animals were killed 90 min later in order to identify properties of proliferating cells without allowing them time to complete a cell cycle. A second group of animals received 4 injections over a 12 hour period in order to be sure all cells that might pass through the S phase of the cell cycle during the 12 hours would be labeled. Animals in this subset were killed 24 hours after the initial injection. For "birth date" studies, at each developmental age some animals that were labeled for a 12 hour period were allowed to survive for an additional 1-2 weeks while the bowel of others was not studied until the 33rd postnatal day. For each experiment the duodenum was removed, fixed appropriately and prepared for radioautography. Some tissues were embedded in Epon and cut at 1 um so that the morphology of dividing cells could be recognized. Additional experiments were done with frozen sections to simultaneously demonstrate $^{3}$H-TdR labeling and serotonin (5-HT) or vasoactive intestinal polypeptide (VIP) immunoreactivity in the same sections. Results indicate that: (1) On the first postnatal day the precursors of some cells that express VIP are still dividing; however, no cells with 5-HT were found to be labeled even by a 12 hour exposure to $^{3}$H-TdR. (2) The murine gut does retain a pool of proliferating neuroblasts, at least until the 2nd week of postnatal life. Neurons continue to be labeled by $^{3}$H-TdR injected up to this time. By one month of age only glia became labeled by $^{3}$H-TdR. Thus proliferation of precursor cells continues throughout or beyond the period when neuronal phenotypic expression occurs. VIP-containing neurons could be derived from the pool of proliferating neuroblasts. Since serotonergic neurons are probably all postmitotic while VIP-containing neurons are still being born, it is unlikely that this peptidergic neuron arises from earlier-developing serotonergic neurons that change their transmitter. Supported by NIH grants NS15547, AM07030 and NSF BNS83-04904.

337.4 ONTOGENY OF SEROTONIN RECEPTORS IN THE MURINE GUT. T. Branchek and M.D. Gershon. Anatomy and Cell Biology, Columbia University, Coll. of P&S, New York, NY 10032.

The enteric nervous system (ENS) is critical in regulating intestinal absorption and motility. In order to gain insight into the factors involved in expression of receptors in the ENS, we investigated the development of a receptor for a transmitter the ontogeny of which is already known, the enteric type of serotonin (5-HT) receptor (5-HTR). 5-HT is a transmitter that acts in both myenteric and submucosal plexuses. The enteric 5-HTR has recently been characterized by radioligand binding studies and shown to be different from either of the major classes of 5-HT receptor that have been found in the CNS. 5-HTR are located on myenteric and submucosal neurons and they are probably also present on primary afferent nerve fibers in the intestinal mucosa. We followed the time course of the elaboration of enteric 5-HTR by radioautographically localizing high affinity $^{3}$H-5-HT binding sites in embryonic and early postnatal mice. Specific binding was defined as that displaced by a 1000-fold excess of 5-HT. Embryos were obtained on gestational days E11-18. Neonatal mice and animals at postnatal days 2,6, 14, and 22 were also examined. Stomach, small and large intestines were dissected in oxygenated Krebs solution, divided into segments, keeping the oral-anal sequence intact, and frozen in OCT embedding compound with liquid nitrogen. Unfixed cryostat sections were cut at 15um, incubated with $^{3}$H-5-HT (in the presence or absence of excess 5-HT), dried, and apposed to $^{3}$H-sensitive film for 2 weeks at room temperature. The developed images were examined for specific binding of $^{3}$H-5-HT. 5-HTR could first be detected on day E14. At this time they were evident in the stomach and the proximal small intestine. One day later, the entire small bowel was found to contain 5-HTR. The proximal colon began to elaborate these receptors by day E16. At day E18, 5-HT receptors extended from the stomach through the proximal colon. Expression of 5-HTR in the distal colon was quite delayed and was not detectable until day P14; the terminal 2-5mm of bowel (measured from the anus) were devoid of detectable 5-HTR until day P22. Serotonergic neurons in the ENS develop very early in relation to other types of enteric neuron and make their appearance in proximodistal sequence from stomach to anus over the course of two days (E12-E14). In contrast, 5-HTR, which also appear in a proximodistal sequence, are elaborated over a later and much longer time course, nearly 4 weeks. The role of 5-HT in regions of the ENS in which the mature type of 5-HTR has not yet appeared is unclear. The appearance of 5-HTR after serotonergic neurons is consistent with the hypothesis that these neurons play a role in inducing the formation of 5-HTR. This hypothesis remains to be tested. Supported by Grants NS12969, NS15547 and the PMAF.

337.5 SYMPATHETIC NERVOUS SYSTEM CONTROL OF PLASMA RENIN ACTIVITY IN DEVELOPING RATS. R. F. Kirby and A. K. Johnson. Dept of Psychology and the Cardiovascular Center, Univ. of Iowa, Iowa City, IA 52242.

Sympathetic nervous system innervation to the heart and adrenal medulla becomes functional during the first postnatal week in the rat. However, the development of sympathetic control of renin release from the kidney has not been examined.

In previous work, doses of tyramine and insulin were found which produced comparable increases in plasma renin activity (PRA) at 30 min post-injection in 20 day old Sprague-Dawley rats. Tyramine was used to directly stimulate norepinephrine release from sympathetic terminals, while insulin was selected because it produces a centrally-mediated activation of the sympathetic nervous system, causing the release of both epinephrine and norepinephrine. In the present study, PRA was measured as an index of functional sympathetic innervation to the juxtaglomerular cells of the kidney in 10 and 15 day old rat pups.

Pups 10 and 15 postnatal days of age received subcutaneous administration of vehicle (distilled $H_2O$), the direct beta-adrenoceptor agonist isoproterenol (0.1 mg/kg), or insulin (20 IU/kg). Pups were decapitated 30 min post-injection and trunk blood was collected into chilled, EDTA treated tubes. Blood samples were then centrifuged and plasma was removed for determining PRA by measuring the formation of angiotensin I in the presence of endogenous angiotensinogen.

Isoproterenol led to large increases in PRA at both ages, indicating that direct beta-adrenoceptor activation is effective in stimulating PRA by 10 days of age. However, tyramine treatment did not produce a significant increase in PRA until day 15. The development of a functional response to tyramine between postnatal days 10 and 15 may indicate that sympathetic terminal mechanisms involved in the uptake and release of norepinephrine mature during this period. Insulin treatment did not stimulate PRA in either 10 or 15 day old pups. Therefore the PRA response to sympathetic nervous system activation with insulin must mature sometime between postnatal days 15 and 20, the age at which our previous dose-response studies had been done.

These results suggest that functional innervation to the juxtaglomerular cells of the kidney matures very late in development when compared to the onset of functional sympathetic nervous system innervation to other target organs, such as the heart and the adrenal medulla.

337.6 PARASYMPATHETIC REGULATION OF THE HEART IN DIABETES. G.O. Carrier and R.S. Aronstam. Department of Pharmacology and Toxicology, Medical College of Georgia, Augusta, GA 30912.

Atria isolated from rat rendered diabetic by exposure to streptozotocin (STZ) are supersensitive to muscarinic agonists (J.Mol. Cell.Cardiol. 16:963, 1984). To further define the nature of this change, the following measurements were made in spontaneously-beating right atria and electrically-stimulated left atria isolated from short-term (10-12 weeks) STZ-diabetic and age-matched control rats: 1) The influence of agonists on chronotropic and inotropic properties, 2) muscarinic receptor number and binding properties, 3) acetylcholinesterase activity, and 4) high affinity [$^3$H]choline uptake.

Atria from STZ-diabetic rats were supersensitive to the negative chronotropic actions of acetylcholine, carbamylcholine and bethanechol. There was no difference, however, in the sensitivity of electrically-stimulated left atria to the negative inotropic actions of the three agonists (Table).

Physiological Responses to Muscarinic Agonists, EC50 Values in uM

| | Chronotropic | | Inotropic | |
|---|---|---|---|---|
| Agonist | Control | Diabetic | Control | Diabetic |
| Acetylcholine | 210 | 15 | 0.41 | 0.28 |
| Carbamylcholine | 0.62 | 0.16 | 0.18 | 0.10 |
| Bethanechol | 10 | 4.6 | 3.0 | 3.2 |

Acetylcholinesterase levels in the right atria from STZ-diabetic rats were lower than in controls (3.59±0.28 vs 5.73±0.83 nmoles/mg protein/min; $p<.05$, N=12). This reduction was accompanied by a 30% decrease in the number of [$^3$H]QNB binding sites. In contrast, there was no change in acetylcholinesterase levels in left atria from STZ-diabetic and age-matched control rats (3.2±0.3 vs 3.8±0.5 nmoles/mg protein/min, respectively).

Uptake of [$^3$H]choline was measured in strips of freshly dissected left and right atria. Neuronal uptake of choline in both right and left atria was not changed in diabetes. Hemicholinium-3 inhibited 39±6% of the uptake in control right atria compared to 41±4% in STZ-diabetic rats. Similarly, in left atria 46±3% and 46±5% of choline uptake could be inhibited by hemicholinium-3 with tissues from control and STZ-diabetic rats, respectively.

These results indicate that cholinergic regulation of heart rate, but not contractility, is altered in the early stages of diabetes. The postganglionic parasympathetic system appears to be intact at this stage of the disease process insofar as choline uptake is not altered. The enhanced sensitivity of right atrium to cholinergic agonists may be related to changes in postsynaptic receptor transduction mechanisms.

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337.7 DEVELOPMENT OF LOW-FREQUENCY VARIATION IN HEART RATE OVER THE FIRST 6 MONTHS OF LIFE DURING SLEEP STATES. V.L. Schechtman, Z. Frostig*, and R.M. Harper. Neuroscience Program, Department of Anatomy, and the Brain Research Institute, UCLA, Los Angeles, CA 90024.

A prominent feature of infant heart rate is rhythmic variation at frequencies of .07 to .12 cycles/sec (periods of approximately 8 to 14 seconds). A variety of mechanisms have been suggested to underlie the source of this low-frequency variation, including circulation time and alterations in blood pressure. Sleep states, particularly REM sleep, dramatically alter blood pressure and peripheral and visceral blood flow. We thus studied state-related influences on low-frequency heart rate variation. Because cardiovascular dynamics change markedly with age, particularly over the first 3 months, we examined developmental characteristics of low-frequency variation in cardiac R-R intervals over the first 6 months of life.

Twenty-five normal infants were polygraphically recorded for determining sleep and cardiac parameters at 1 week and at 1, 2, 3, 4, and 6 months of age. Variations in heart rate were extracted from R-R intervals by a peak/trough detection algorithm (Frostig, Z., et al., Soc. Neurosci. Abs., 1985) and variations with periods ranging from 8.3 to 14.3 seconds were examined. The median amplitude of this variation was determined for each minute, and the median of these values was calculated for quiet sleep (QS) and REM sleep for each age.

The amplitude of low-frequency heart rate variation showed a pronounced drop at 1 month in both QS and REM. That decline, however, was largely dependent on the concomitant rise in heart rate. Overall heart rate greatly affected the amplitude of variation, especially after 1 week. After partitioning heart rate contributions, covariance analysis revealed that the amplitude of variation was higher in REM than in QS at every age, and gradually declined with age during REM.

The extent of low-frequency heart rate variation is altered by many factors, including development, sleep state, and heart rate.

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337.8 TYROSINE HYDROXYLASE ACTIVITY IN ADRENAL AND HEART: EFFECTS OF PARTIAL DAMAGE AND STRESS. G.L. Snyder*, S.J. Fluharty*, L.E. Rabow*, E.M. Stricker, and M.J. Zigmond. Psychobiology Program and Center for Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA 15260. (Spon: David C. Wood)

Adrenal tyrosine hydroxylase (TH) activity is elevated by stress and by partial damage to sympathetic nerves. Increased TH activity probably functions to couple catecholamine (CA) synthesis with increased demands for adrenal CA secretion under both conditions. In the present experiments, we studied the impact of unilateral adrenalectomy (UAX) on TH activity in the remaining adrenal medulla and cardiac sympathetic nerves during stress.

Four days after UAX in adult male Sprague-Dawley rats, adrenal TH activity was found to be significantly elevated (by 44%). 6-Hydroxydopamine (6-HDA) treatment (100 mg/kg, s.c.), which by itself increased TH activity by 68%, raised it by 112% in rats with UAX. As indicated in the Table below, there was a further increase in TH activity when rats either were exposed to cold (4 days at 4°C) or were given 20 U/kg insulin and killed 1 hr later.

The TH activity of cardiac sympathetic nerves also was elevated by UAX (by 43%), and it was further increased by cold and insulin treatments (see Table). Denervation of the contralateral adrenal gland abolished the effect of UAX on adrenal TH activity but had little additional effect on basal cardiac TH activity, although it did have an additive effect on cardiac TH activity when UAX rats were exposed to chronic cold stress. These results illustrate that CA synthesis is elevated both in adrenal medulla and sympathetic nerves after UAX, as after subtotal damage to the sympathetic nerves, presumably in response to some functional consequence of the loss of circulating CA. Nevertheless, the capacity for CA synthesis can be raised still further by stress.

Supported by MH-29670 and NS-19608.

TH Activity (% basal control)

| | ADRENAL | | CARDIAC | |
|---|---|---|---|---|
| | Cold | Insulin | Cold | Insulin |
| Control | 193 ± 10 | 135 ± 11 | 170 ± 17 | 132 ± 7 |
| UAX | 250 ± 5 | 198 ± 5 | 173 ± 10 | 176 ± 14 |
| 6-HDA | 267 ± 11 | 202 ± 6 | | |
| UAX + 6-HDA | 434 ± 5 | 293 ± 12 | | |

337.9 THE EFFECTS OF NEONATAL GUANETHIDINE TREATMENT ON THE ADRENAL MEDULLA OF THE RAT. L.L. Ross, A. Pylypiw*, L. McCarthy* and L. Cosio*. Dept. of Anatomy, The Medical College of Pennsylvania, Philadelphia, PA 19129.

Chronic administration of guanethidine to some strains of developing rats during the first 3 weeks of life has been reported to produce a total and irreversible destruction of sympathetic postganglionic neurons. Both parasympathetic postganglionic neurons and adrenal chromaffin cells are apparently unaffected by this treatment. We have previously shown that accompanying this peripheral loss is a parallel loss of preganglionic neurons in the thoracolumbar spinal cord. Since the same pool of spinal cord neurons provides preganglionic innervation to sympathetic neurons and to the adrenal medulla, we examined the effects of chronic guanethidine treatment on the adrenal medulla and its preganglionic innervation.

Guanethidine (50mg/kg/day) was administered to neonatal Sprague-Dawley rats 5 days/week for 3 weeks starting at 3 days of age. The degree of sympathectomy at 25 days of age was shown by the absence of norepinephrine (NE) in the spleen and in the heart while gut levels of NE were reduced to 15% of control values. On the other hand, adrenal levels of NE and epinephrine (E) were 50% and 25% greater than controls, respectively. The degree of hyperactivity of the adrenal medulla was further demonstrated by the observation that plasma NE levels were down by only 15%. Normally, the adrenal medulla provides only 10% of the circulating NE. Although adrenal choline acetyltransferase (CAT) was elevated by 40% in the sympathectomized animals, there was no increase in the numbers of preganglionic neurons projecting to the adrenal medulla as determined by true blue retrograde labeling. The most striking response to neonatal guanethidine treatment is by the adrenal chromaffin cell itself, whose mitotic activity was 5x that of controls, resulting in a 75% increase in the number of chromaffin cells at 25 days of age. This increase is not accompanied by a significant increase in the size of the adrenal medulla. Thus, the adrenal medulla compensates for the guanethidine destruction of sympathetic neurons by cell proliferation and increased synthesis of NE and E. This is accomplished without any enhancement of central innervation, an indication of the great degree of plasticity of the developing chromaffin cell and its ability to compensate for decreased circulating levels of NE.

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337.10 LOSS OF $Ca^{2+}$ SENSITIVE $K^+$ CONDUCTANCE MAY INCREASE $Ca^{2+}$ INFLUX INTO AXOTOMIZED SYMPATHETIC NEURONES. M.E.M. Kelly*, J. Shapiro*, T. Gordon & P.A. Smith. Department of Pharmacology, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7.

Neuronal cell bodies undergo various physiological changes following section of their axons. These changes may be related to the neurones ability to regenerate and re-innervate its target organ. Transection of the axons of soleus motoneurones results in shortening of the action potential after-hyperpolarization (AHP, Kuno et al. J. Physiol 240, 725, 1974). We used standard intracellular recording techniques to test whether axotomy produced similar changes in another neuronal type; B cells in bullfrog sympathetic ganglia. As in slow motoneurones, the duration of the antidromically evoked action potential AHP was reduced from 147.6±15.1 msec (n=27) to 47.2±2.4 msec (n=47), 14-47 days after section of the post-ganglionic nerve. The amplitude of the response was reduced from 29.5±0.7 mV (n=27) to 18.2± 0.8mV (n=47). In addition, the duration of the action potential was increased from 3.4±0.1 msec (n=24) to 4.4±0.2 msec (n=47). All changes were significant ($P<0.001$). The $Ca^{2+}$ channel blockers $Cd^{2+}$ (200 μM) or $Mn^{2+}$ (10 mM) produced similar effects by blocking the $Ca^{2+}$ sensitive $K^+$ conductance(s) ($g_{K,Ca}$) which underlie part of the AHP and which may also contribute to the repolarization of the action potential (Adams et al. Nature 296, 746, 1982, MacDemott & Weight, Nature 300, 185, 1982). $Cd^{2+}$ or $Mn^{2+}$ did not shorten the AHP of axotomized cells. Despite this, $Ca^{2+}$ spikes could be evoked in these cells in the presence of 10 mM TEA/2 μM TTX. This implies that $Ca^{2+}$ channel function was preserved after axotomy but the $g_{K,Ca}$ which underlies part of the AHP may be lost. Such a response to axotomy might be expected to enhance $Ca^{2+}$ influx as a result of spike broadening (cf MacDermott & Weight, Nature 300, 185, 1982). Increased intracellular $Ca^{2+}$ could then activate further processes which may be related to regeneration.

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337.11 DEVELOPMENTAL REGULATION OF NEURON NUMBER IN A PARASYMPATHETIC GANGLION. R. David Heathcote and Peter B. Sargent. Department of Cell Biology, Stanford University School of Medicine, Stanford, CA. 94305.

The regulation of neuronal number is presumed to play a role in the matching of populations of neurons with their targets. Little is known about the mechanisms which regulate the proliferation and death of neurons and thus control their number. We have studied the regulation of neuronal number in an autonomic ganglion whose neurons are generated over an extended period of time and do not undergo detectable cell death. The cardiac ganglion of the frog Xenopus laevis is derived from the embryonic neural crest. Differentiated neurons can be selectively stained for acetylcholinesterase and begin to appear in the heart during embryogenesis. Approximately 30 neurons are present at the end of the first week of development and approximately 1300 are present in the adult. The prolonged period of neuron addition has allowed the use of various manipulations designed to examine the regulation of neuronal number.

One possible regulative mechanism is that the increase in neuron number is controlled by an internal clock such that a defined number of neurons is added at regular intervals. However, by raising animals of the same chronological age under different conditions, it is possible to introduce as much as a three-fold difference in neuronal number. Those animals with more neurons are developmentally more advanced and larger than their siblings. To distinguish between developmental stage and size, animals were arrested at a particular developmental stage by blocking thyroxine production with propylthiouracil. These animals continue to increase in size, and the number of neurons in their cardiac ganglia increase many-fold until the approximate number of adult neurons is attained. Thus neither a chronological or developmental clock times the addition of neurons to the ganglion. However the above results are consistent with animal and target size playing a role in the regulation of neuronal number.

During development the size of cardiac neurons changes as well as their number. Between one week of development and the end of metamorphosis, the average size actually decreases due to the addition of many small neurons. As postmetamorphic adults increase in size, their neurons also get larger. Animal size increases by over four orders of magnitude and is highly correlated with the product of neuronal size and neuronal number. The relationship is a power function of the form $y=bx^m$ and allows one to predict the total neuronal volume of a ganglion if the size of the animal is known.

The growth of the cardiac ganglion can be divided into three overlapping segments. First, a dowry of neurons is provided by the neural crest. Second, neurons are added to the ganglion. This addition is correlated with the size of the animal and its target and does not occur at specific times or developmental stages. Third, once the adult number of neurons is reached, neurons increase in size. In all three phases, the relationship between total neuronal volume and the size of both the target tissue and the animal is maintained.

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337.12 CATECHOLAMINERGIC INNERVATION OF THE PANCREAS IN THE AGED MOUSE: PRELIMINARY OBSERVATIONS. B.J. Davis, J.A. Orsini* and T.H. McNeill. Department of Neurology, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642.

Pancreatic islets are innervated by autonomic nerve fibers that may play a role in maintaining islet function and growth. The purpose of the present study was to determine whether normal islet innervation patterns are maintained during senescence using the C57BL/NNia mouse as an animal model.

Pancreata were obtained from five 6-month-old (young adult) and six 30-month-old (senescent) male mice. Samples of the head, body and tail of the pancreas were fixed in Bouin's fluid then dehydrated and embedded in a single paraffin block. Serial 4 um sections obtained at 200 um intervals through the tissue block were stained for immunocytochemical localization of insulin (B), glucagon (A), somatostatin (D) and pancreatic polypeptide (PP) cells using the avidin-biotin (Vectastain) method. Additional samples of pancreas from each animal were processed for catecholamine histofluorescence using the sucrose-phosphate-glyoxylic acid (SPG) technique. Tissues from young and senescent animals were processed concurrently to minimize variability due to differences in handling.

Morphometric analysis revealed that when compared with young adult mice, the senescent mice showed an increase in the total amount (volume density) of islet tissue in the pancreas. While the quantitative relationships of the A, B, D and PP cells were similar between the two age groups, senescent mice tended to show an increase in the relative proportion of B cells within the islets. Fluorescence histochemical analysis revealed a striking increase in autofluorescent lipofuscin-like material associated with the cytoplasm of exocrine and endocrine cells in the pancreas of senescent mice. Lipofuscin-containing neuronal perikarya also were seen in the senescent, but not in the young pancreas. Catecholamine-containing nerve fibers appeared coarser and more intensely fluorescent and were more densely distributed in some areas of the senescent pancreas.

These preliminary results suggest that catecholaminergic innervation of the pancreas shows considerable plasticity, but is well maintained during aging.

338.1 MOTOR INNERVATION OF CAT'S DIAPHRAGM: RELATION TO VOMITING. L.K. Tan and A.D. Miller. Rockefeller University, NY, NY 10021.

Vomiting is primarily produced by co-ordinated action of the respiratory muscles. Most of the diaphragm contracts during both retching and expulsion; however, the region around the esophagus is virtually inactive during expulsion, thereby facilitating rostral movement of gastric contents (2). We report our studies of (i) localization of motoneurons supplying these different regions of the diaphragm, including an extra-phrenic innervation, and (ii) the functional significance of this extra-phrenic motor innervation.

Horseradish peroxidase was injected into the diaphragm within 0.7 cm of the esophagus in 2 cats (35-50 µl of 30% HRP), using Nembutal anesthesia and aseptic procedures. Four days later, the animals were sacrificed and tissue reacted with TMB. Labeled motoneurons were found bilaterally over a widespread region from caudal C4-rostral C7 and from T5-L1. Injections (65 µl) into the region of the diaphragm that is active during expulsion (> 1 cm from the cat's esophagus) in a third animal resulted in labeled motoneurons from caudal C4-rostral C7 and from T2-L1. More labeled neurons were observed in the thoracic cord than in the phrenic pool. Control injections were made in the thoracic (10 µl) and abdominal cavities (20-55 µl) in 2 cats to ensure that thoracic labeling was not due to spread of HRP.

In a separate series of experiments involving decerebrate cats, EMG electrodes were sewn into different regions of the diaphragm, and nerve activity was recorded from the contralateral C5 phrenic nerve and upper lumbar abdominal nerves. Vomiting was produced by electrical stimulation of the sub-diaphragmatic vagus nerve (1). Latencies to expulsion averaged somewhat less than 1 min. After cutting all phrenic nerves at the cervical level in 2 cats, respiratory-related diaphragmatic EMG activity was abolished or greatly reduced; remaining EMG activity could be either inspiratory, expiratory, or phase-spanning (cf.3). In contrast, latencies to expulsion were virtually unchanged, and the diaphragm continued to be phasically activated during vomiting, at 20-80% of its amplitude prior to phrenic nerve section.

These studies reveal a large motor innervation of the diaphragm from thoracic levels and show that these thoracic motoneurons can participate in the control of the diaphragm during vomiting.

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338.2 ELECTRICAL STIMULATION IN THE EXTERNAL CUNEATE NUCLEUS: EFFECTS ON RESPIRATION. C. Dean* and D.R. Kostreva (SPON: J.P. Kampine) Dept. Anesth., Med. Col. Wis. and VAMC, Wood, WI 53193

[$^{14}$C]2-deoxyglucose studies have suggested that the external cuneate nucleus is involved in cardiovascular control (Kostreva Physiologist 26:333-350,1983). Recent experiments in anesthetized cats in our laboratory have shown that electrical stimulation within the external cuneate nucleus can evoke profound changes in arterial blood pressure, heart rate, efferent renal and cardiopulmonary sympathetic nerve activity (Dean & Kostreva Fed. Proc. 44: 1029 (3616), 1985). Such stimulation can also elicit changes in respiration. Experiments were carried out on spontaneously breathing cats anesthetized with sodium pentobarbital (Nembutal 35 mg/kg i.v.) in which arterial blood pressure, heart rate and the electromyography of the diaphragm were recorded. Electrical stimulation was effected using indium/ Woods metal filled glass microelectrodes, with a tip diameter of 10-20 um. The stimulus parameters were 50 uA, 0.1 ms duration, 5-100 Hz. Stimulation of localized sites within, and immediately adjacent to the external cuneate nucleus could increase the duration of inspiration, the duration of expiration and the depth of inspiration. These effects were observed simultaneously with the pressor or depressor responses, in addition to the bradycardia or tachycardia previously identified during external cuneate nucleus stimulation. At some sites respiratory responses lasted three times longer than did the cardiovascular responses. The evoked responses were frequency dependent as decreasing this stimulus parameter could change the response elicited, for example, from an increased expiratory period and diminished depth of inspiration to an inspiratory apnea. Stimulus sites were subsequently identified histologically. These results indicate that in addition to an involvement in cardiovascular control, the external cuneate nucleus may also be involved in the control of respiration. Considering the generally accepted view that the external cuneate nucleus is a system for transmission of information from the forelimbs and trunk to the cerebellum, perhaps a further role for this nucleus is in the mediation of somato-autonomic and somato-respiratory reflex responses in such circumstances as exercise. (Supported by NIH Grants HL27968, NS18037, RCDA HL00959, and the VA).

338.3 MEDULLARY BULBOSPINAL EXPIRATORY NEURON RESPONSE TO INTERCOSTAL MUSCLE TENDON ORGANS. R. Shannon, D.C. Bolser* and B.G. Lindsey. Dept. Physiology, Col. Med., Univ. So. Florida, Tampa, FL 33612.

Previous studies from this laboratory provided evidence that external and internal intercostal muscle tendon organs have (a) a generalized inhibitory effect on medullary dorsal and ventral respiratory group inspiratory neurons driving the inspiratory muscles (diaphragm, intercostals and laryngeals), and (b) an augmenting effect on medullary expiratory laryngeal motoneurons. The objective of this study was to determine the effects of intercostal tendon organs on ventral respiratory group expiratory neurons which control expiratory intercostal and abdominal muscle activity.

Studies were conducted on decerebrate, vagal intact and vagotomized, mechanically ventilated cats (six). Tendon organs in the T6 intercostal muscles were stimulated by impeded muscle twitches, elicited by electrical stimulation of the peripheral end of cut T6 ventral roots (VRS). The T6 space was surgically separated from adjoining spaces and all muscles other than those being studied were denervated. Medullary expiratory neurons were extracellularly recorded from the caudal ventral respiratory group (below obex) and identified as bulbospinal by antidromic stimulation (positive collision test) of the cord at T1. Phrenic efferent activity was also monitored. The strength of the impeded twitches was adjusted by VRS to produce the maximum change in phrenic activity (transient reduction or complete termination), and then the response of expiratory neurons was determined. Impeded twitches during the expiratory phase caused a transient reduction in firing rate in 17 of 33 E-neurons (8/17 were identified as bulbospinal).

Combining these results with previous studies on expiratory neurons, in which electrical stimulation of intercostal nerve Group I and II afferent fibers showed similar effects and muscle spindle ending stimulation by vibration had no effect on medullary respiratory activity, we conclude the following about external and internal intercostal tendon organs: (1) They can reduce the activity of some medullary bulbospinal expiratory neurons, and at the same time extend the duration of the expiratory phase. (2) They have different effects on the medullary control of expiratory laryngeal muscles and expiratory bulbospinal neurons. (Supported by NIH Grant HL-17715).

338.4 EFFECTS OF LESIONS OF NUCL. PARABRACHIALIS MEDIALIS AND OF BARBITURATE ON PHRENIC AUGMENTATION RATE AND PHRENIC FACILITATION BY LUNG INFLATION. W.R. See*, M.I. Cohen and A.L. Sica*. Dept. of Physiology, Albert Einstein College of Medicine, Bronx, NY 10461.

Phrenic augmentation rate and the degree to which it is facilitated by lung inflation is dependent on central nervous system state. We have recently demonstrated that elevation of $CO_2$ tends to increase the excitatory effect of lung inflation on phrenic discharge (Fed. Proc. 44(5): 1586, 1985). In the present series we investigated the influence of unilateral lesions of the Nucl. parabrachialis medialis (NPBM) and of small i.v. doses of pentobarbital (1.8-2.6 mg/kg) on the phrenic response to lung inflation. Experiments were performed in unanesthetized, paralyzed, decerebrate cats ventilated by a cycle triggered pump. The effects of lung inflation on the magnitude and augmentation rate (slope) of phrenic activity were ascertained by comparison of activity during equivalent time periods in inflation (control) vs. no-inflation (test) inspiratory phases under different conditions (low and high $CO_2$, pre- and post- pontine lesion, and pre- and post- barbiturate). The effects of lung inflation were classified as facilitatory (+), depressant (-), or not significant (0), using the ratios of phrenic activity and slope between test and control cycles. Barbiturate or unilateral lesions of the NPBM reduced the slope of phrenic activity in both inflation and no-inflation cycles but to a lesser extent in no-inflation cycles, which resulted in a reduction or complete disappearance of the (+) response and in some cases the conversion of a (0) response into a (-) response. In conclusion, in contrast to the effect of $CO_2$, which increases the excitability of the inspiratory ramp generator to lung receptor inputs, barbiturate or lesions of the NPBM reduce this excitability.
(Supported by USPHS Grant HL-27300 and DFG Grant SFB 114.)

338.5 EVIDENCE FOR MECHANISMS OF ACTIVATION AND ANALAGOUS ACTIONS OF DECREMENTING VENTRAL RESPIRATORY GROUP NEURONS AT INSPIRATORY AND EXPIRATORY PHASE TRANSITIONS. L.S. Segers*, R. Shannon, and B.G. Lindsey (SPON: S. Saporta). Dept. Physiol., Univ. South Florida Med. Ctr., Tampa, FL 33612

Data from this laboratory (1) are consistent with the idea that E/IE cells with decrementing activity in the E phase play a role in I-E phase switching and in control of augmenting E neuron activity. Here we report additional observations in support of this hypothesis, data consistent with the hypothesis that decrementing early I cells serve analagous functions at the E-I phase transition and in control of augmenting I activity, and additional data relevant to the mechanisms contributing to the activation of these cell types. Methods were as described elsewhere (1); responses of cells to vagal or internal intercostal ($T_7$) nerve stimulation were noted in some cases. The stimulation studies support the idea that E/IE cells help to control the ramp of augmenting E activity via inhibitory effects on augmenting E cells (1): both an increase in E/IE and a decrease in augmenting E cell activities have been observed following vagal stimulation late in the I phase. Cycle triggered histograms (CTHs) show that the average time course of the decline in rate of a decrementing early I cell is the reciprocal of that of the average rate increase of simultaneous phrenic efferent activity. Cross-correlograms (CCHs) and CTHs generated for simultaneously recorded augmenting E and decrementing early I cells show that activities of these two cell types may overlap, a finding consistent with the hypothesis that decrementing early I cells may contribute to the termination of the E phase by inhibiting augmenting E cells. CCHs consistent with inhibition of augmenting I cells by decrementing early I cells and with excitation of decrementing early I cells by EI cells were also obtained. CTHs generated for sequentially recorded neurons in apneustic animals indicate that the durations of the E phase and decrementing early I cell activity can be independent of the duration of the I phase and support the idea that E/IE cells receive inhibitory inputs throughout the I phase (1). E/IE cells are silent during the first part of the I phase, corresponding to decrementing early I cell activity, after which they are active at a low rate until the I-E phase transition. Furthermore, vagal stimulation during the I phase can inhibit phrenic activity before E/IE cell activity increases. Finally, cycle triggered scatter diagrams generated for simultaneously recorded decrementing E cells show that the cells are most strongly synchronized at the start of the E phase. This is consistent with coincident disinhibition. In summary, our data support the hypothesis that decrementing I and decrementing E cells each contribute to the termination of the preceding phase of the cycle and are involved in the development of augmenting respiratory neuron activity in their respective phases. Reference: 1. Lindsey, B.G., et al., these proceedings. Support: NIH Grant NS19814.

338.6 FUNCTIONAL ASSOCIATIONS AMONG VENTRAL RESPIRATORY GROUP (VRG) NEURONS IN THE CAT. IMPLICATIONS FOR CENTRAL PATTERN GENERATION. B.G. Lindsey, L.S. Segers*, and R. Shannon. Dept. Physiol., Univ. South Florida Med. Ctr., Tampa, FL 33612

Interactions among brainstem respiratory neurons are believed to be necessary for respiratory rhythmogenesis. However, these associations are not well understood. We have reported (1) evidence for functional interactions among various types of VRG (region of n. ambiguus, n. retroambigualis, retrofacial n.) neurons based on spike train correlation methods. Proposed interactions included: 1. excitation of bulbospinal (b.s.) $I^{\dagger}$ neurons by propriobulbar (p.b.) I cells, 2. inhibition of p.b.E/IE cells by decrementing p.b.I cells, 3. inhibition of I/EI cells by some E/IE cells, and 4. excitation of augmenting E cells by other E/IE cells. Here we report further associations among ipsilateral VRG cells. Cats (n=31) were anesthetized, paralyzed, vagotomized and artifically ventilated. VRG neurons were recorded with 4-6 tungsten electrodes. Phrenic efferent activity was monitored. Cycle triggered histograms were generated; most neurons were tested for spinal and vagal projections by antidromic stimulation methods. 467 pairs of neurons were analyzed; 16% were correlated in a manner indicative of cross-connections or shared inputs. Cross-correlograms suggesting the following additional associations were obtained: 1. excitation of p.b.I cells by p.b.I/EI cells, 2. inhibition of p.b. augmenting E cells by p.b.E/IE cells and 3. inhibition of E cells by tonic cells. These and other data from this laboratory (1,2,3) provide evidence for network interactions which could play a role in central pattern generation. Working hypotheses include: 1. E/IE cells inhibit inspiratory drive late in the I-phase as their activity increases due to reduced decrementing I cell activity, (i.e., disinhibition), which in turn may be due to central and afferent mechanisms operating in parallel. 2. E/IE cells also inhibit augmenting E cells. 3. The inhibitory actions of E/IE cells decline during the E-phase, promoting augmenting E cell activity and eventually releasing EI and I cells from inhibition. 4. P.b.I/EI cells drive p.b. I cells, some of which may drive b.s. pre-motor neurons.

$^{\dagger}$Phase of maximum firing rate: I=inspiratory, E=expiratory. Phasic cells active during more than one phase have second descriptor, e.g., E/IE denoting phase transition during discharge.

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338.7 CHARACTERISTICS OF VAGAL AFFERENT CONTROL OF THE DISCHARGE PATTERNS OF MEDULLARY RESPIRATORY NEURONS. E.J. Zuperku* and F.A. Hopp*. (SPON: J. Seagard). Dept. of Anesth., Med. Col. Wisc. and the VAMC, Wood, WI 53193.

The central processing of vagal afferent input patterns which play a role in the control of breathing was studied by analyzing the responses of individual respiratory neurons to these inputs. Unit recordings were obtained from both inspiratory (I) and expiratory (E) neurons in the medulla of halothane anesthetized, paralyzed, artifically ventilated, bivagotomized dogs. Phrenic nerve activity was used as a timing reference and to measure I and E phase durations($T_I$ & $T_E$). Electrical activation of the largest vagal A fibers was used to alter $T_I$ and $T_E$ and present various temporal input patterns to I and E neurons. Discharge patterns were quantitated using cycle triggered histograms (CTHs). The net neuronal response to vagal input patterns was measured as the difference between input ($H_s$) and no input (control, $H_c$) CTHs or $H_d=H_s-H_c$. In the majority of neurons, the following observations, which are independent of type (I or E) and pattern (augmenting or decrementing), were made: 1) the difference histogram ($H_d$) is zero at the onset of the input and is either a monotonically increasing or decreasing function of time; 2) for I-neurons, the step response time from onset of stimulus to 90% of max response was greater than 300 msec; for E-neurons, this response time was greater than 1 sec.; 3) the vagal pulse-to- spike interval histograms show no or a small, broad, delayed increase in the probability of neuronal firing; 4) among neurons the shape of the $H_d$ time-course appears not to be consistent, varying from convex-to-linear-to-concave. In a given neuron the shape varies with step frequency; 5) plots of $H_d$ vs $H_c$ or $H_s$ appear to be linear with r>.8; this implies that: $H_s=\beta \cdot H_c+\alpha$ (where $\beta$ is slope, $\alpha$ is intercept); 6) for a given step input, the time course of $H_c$ and $H_s$ can be predicted from that of $H_d$ with a mean error of less than 10%; and 7) for graded shortening of $T_I$ by step vagal inputs, the plots of $(1/\beta)$ vs $T_I$ are linear. A similar relationship appears to exist for $T_E$. In conclusion, the vagal modulation of respiratory neuronal discharge patterns appears to be directly related (proportional) to the time-course of the control (no input) discharge pattern. This modulation appears to be mediated via the phase timing mechanisms. In addition, these results suggest a possible multiplicative interaction involved with the vagal control of phase timing. (Supported by the VA Medical Ctr.)

338.8 POSSIBLE SITES FOR TACHYPNEIC ACTION OF THYROTROPIN RELEASING HORMONE (TRH)

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Thyrotropin releasing hormone (TRH) has previously been shown to possess potent respiratory stimulating properties (1,2). The tachypnea following intracerebroventricular (i.c.v.) TRH administration produces both a shortening of the inspiratory time and an increase in respiratory frequency. Recently we have shown that the raphe obscurus in the rat medulla is a very sensitive locus for TRH-induced shortening of inspiratory time. The tachypneic response can be blunted by decerebration at the collicular level of unanesthetized rabbit pups (3). We have now attempted to localize sites rostal to the collicular level which are associated with the tachypneic response to TRH in halothane anesthetized rats.

Cannulae guides were implanted at least 48 hours prior to the experiments. Arterial blood gas tensions, blood pressure, heart rate and ventilatory parameters were continuously determined during the experiment. TRH was administered i.c.v. (0.5-5µg/15µl) or locally (100ng/0.5µl). Local injection of TRH (100ng) did not reduce Ttot after injection into the raphe nuclei, the septal region, cerebral cortical areas, cerebellum, amygdala, n. accumbens, or lateral ventricle. However, injection of TRH (100ng) into the posterior hypothalamus, the interpeduncular nucleus and to a lesser extent the habenula nuclei resulted in a tachypneic response similar to that seen somewhat later after i.c.v. administration of higher doses of TRH (1µg). Blood pressure and heart rate changes were small and slight with decreases as well as increases seen.

The present results indicate that the tachypneic response seen after i.c.v. TRH administration to rats involves diencephalic sites. Increased ventilation following activation of these sites might be closely related to the well known arousal effects of TRH. The respiratory stimulating effect after excitation of the ventricular activating system by hypothalamic electrical stimulation (see e.g.[4]) might be of a similar nature.

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338.9 SEROTONIN-MEDIATED EXCITATION OF RECURRENT LARYNGEAL NERVE MOTONEURONS ELICITED BY STIMULATION OF THE RAPHE OBSCURUS. J.R. Holtman, Jr., T.E. Dick, and A.J. Berger. Dept. of Physiology and Biophysics, Univ. of Washington Sch. of Med., Seattle, WA 98195.

Evidence is available suggesting that serotonin and the raphe nuclei may be involved in central respiratory control. Serotonin is present within nuclei known to be involved in respiratory control (Steinbusch, Neuroscience 6: 557, 1981; Maley and Elde, Neuroscience 7: 2469, 1982; Holtman et al., J. Neuroscience 4: 1064, 1984) and stimulation of the raphe nuclei affects overall ventilation (Sessle et al., Brain Res. 216: 145, 1981; Holtman et al., Soc. Neurosci. Abstr. 10: 708, 1984). We have previously investigated the effects of electrical stimulation of the raphe obscurus (RO) and raphe pallidus (RP) on short-latency averaged responses in the phrenic nerves. Stimulation within the RO produced a delayed excitatory response that was reduced by methysergide, a serotonin receptor antagonist (Fed. Proc. 44: 1585, 1985). In the present study, we have extended this work to examine the effects of stimulation of the RO and RP on short-latency averaged responses in the recurrent laryngeal nerve (RLN) and determined if the elicited responses involved serotonin.

Recurrent laryngeal nerve activity was recorded in nine chloralose-urethane (30 and 150 mg/kg) anesthetized, paralyzed (gallamine, 5 mg/kg/hr) and artifically ventilated cats (2-4 kg). Electrical stimulation (2.5-80 μA, 5-80 Hz, 150 μs pulse duration) of the RO and RP was performed using monopolar electrodes. The short-latency responses in the RLN were processed using signal averaging techniques (40 μs/bin, 1024 bins, 200 sweeps).

Analysis of the post-stimulus triggered averages revealed a maximal excitatory response in the RLN produced by stimulation within the RO. The latency to onset for this response was 6.0±0.3 ms. The latency to the peak of this response occurred at 7.8±0.4 ms. This excitatory response was present when the stimulation of the RO was performed during inspiration as well as during expiration. The magnitude of the excitatory response was directly related to current intensity and was also dependent upon stimulus frequency. The threshold stimulus current was 5 μA. Intravenous administration of methysergide (0.1-1.2 mg/kg) resulted in a dose-dependent reduction in the magnitude of the excitatory response.

These results demonstrate that stimulation within the RO produces an excitatory response in the RLN, which is mediated in part by the activation of a serotonergic pathway. The characteristics of the excitatory response in the RLN and the delayed excitatory response in the phrenic nerves are similar. Both responses were: 1) dependent upon stimulus current and frequency, 2) elicited by stimulation during inspiration and expiration and 3) reduced in magnitude by methysergide. (Supported by NS 14857, HL 30849 and the Puritan-Bennett Foundation.)

338.10 ACUTE NERVE AGENT TOXICITY: TIME-COURSE OF RESPIRATORY AND CARDIOVASCULAR EFFECTS. E.T. Beers*, J.F. Glenn, B.L. Perrone*, C. Opalka*, and D.L. Rickett (SPON: R. Ray) Neurotoxicology Branch, US Army Medical Research Institute of Chemical Defense, APG, MD 21010-5425.

While respiratory failure is the common end-point of acute poisoning by organophosphate compounds, the respiratory and cardiovascular systems experience a variety of toxic symptoms prior to respiratory arrest. In the present study, four organophosphate nerve agents (soman, sarin, tabun, and VX) were compared to identify the relative significance of their toxic effects on the central and peripheral nervous system components of the respiratory system and on selected cardiovascular variables. Using DIAL (70 mg/kg, IP) anesthetized cats, recordings were made of i) medullary respiratory-related unit activity, ii) phrenic nerve activity, iii) diaphragm electromyographic activity (EMG), iv) diaphragm contractions, v) airflow, vi) femoral arterial pressure, and vii) electrocardiographic activity; also blood gases and expired $CO_2$ were monitored. The agents were infused at the rate of 1 $LD_{50}$ per 15 minutes until cessation of spontaneous respiration, at which time the phrenic nerve was stimulated supramaximally to test diaphragmatic contraction. Agent infusion then was resumed at 3 times the initial rate, while diaphragm contraction was tested periodically. Of the variables recorded, respiratory-related unit activity is the most sensitive indicator of agent-induced respiratory distress. Unit activity consistently becomes disrupted prior to changes in the other variables. This is followed by changes in phrenic nerve activity, diaphragm EMG, respiratory rate, airflow and heart rate. In addition, blood pressure frequently exhibits a biphasic response, with an increase in pressure followed by a decrease to below control values just prior to respiratory arrest. Moreover, cessation of spontaneous respiration precedes cardiovascular collapse, even though the animal's cardiovascular system becomes increasingly compromised. At the time of respiratory arrest, arterial blood pressure is 82% of control for soman, 74% for tabun, 55% for sarin, and 48% for VX. Similar effects are seen for heart rate. However, the diaphragm muscle still contracts tetanically when challenged with a 100 Hz train of 2 msec pulses for 500 msec at the time of respiratory arrest. Therefore, loss of central respiratory drive results in respiratory failure. Our findings support previous studies in barbiturate-anesthetized animals showing that the central nervous system is most sensitive to nerve agent intoxication and that agent-induced respiratory arrest results from loss of central respiratory drive (de Candole et al., 1953).

338.11 PHARMACOLOGY OF FICTIVE BREATHING IN THE ISOLATED LAMPREY BRAIN. C.M. Rovainen and D.F. Russell. Dept. of Cell Biology & Physiology, Washington University Medical School, St. Louis, MO 63110.

The pharmacological modulation of respiratory bursting was tested in isolated preparations of the lamprey brain or medulla. The experimental advantages include a neuropharmacology similar to that of higher vertebrates, penetration of the nervous tissue by most bath-applied drugs, and spontaneous respiratory bursts in cranial IX-X motoneurons. Adult lampreys of 4 species were used: Ichthyomyzon unicuspis, Lampetra fluviatilis, Lampetra lamottenii, and Petromyzon marinus.

Inhibitory amino acids: 0.2-0.5 mM glycine or GABA reduced the amplitudes and frequencies of respiratory bursts. Strychnine and picrotoxin blocked these effects but did not prevent fictive breathing (Neurosci. 10: 875-882).

Excitatory amino acids: 0.1-1 mM N-methyl-aspartate (NMA), 50 μM kainate, or 2 mM D-glutamate disrupted breathing and produced steady firing of respiratory motoneurons. Lower concentrations produced spontaneous EPSPs and tonic firing in motorneurons, with superimposed respiratory bursts and subsequent pauses. Alpha-aminoadipate at 1-2 mM or 2-amino-5-phosphonovalerate at 100 μM blocked excitation by NMA but not by kainate. Neither antagonist blocked fictive breathing in adult I.u.. However, the less specific antagonist cis-2,3-piperidine dicarboxylate at 2 mM did block fictive breathing as well as the excitation by NMA or kainate.

Substance P: In adult L.f., 1 μM had little effect, 10 μM increased the frequency and burst amplitude of respiration, and 100 μM disrupted breathing and produced sporadic intense 1 s discharges of n.X motorneurons.

Amines: Serotonin at 1-10 μM reversibly depressed the frequency of respiration. Norepinephrine at 1-10 μM dramatically increased the frequency and burst amplitude of fictive breathing in all the species tested.

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338.12 CONNECTIVITY OF SLOWLY ADAPTING PULMONARY STRETCH RECEPTORS (PSRs) TO DORSAL RESPIRATORY GROUP (DRG) NEURONS REVEALED BY INTRACELLULAR SPIKE-TRIGGERED AVERAGING (STA). A.J. Berger and T.E. Dick. Department of Physiology & Biophysics, University of Washington School of Medicine, Seattle, WA 98195.

Lung inflation influences the behavior of DRG inspiratory (I) neurons. It reduces the excitability of DRG Iα cells and facilitates both Iβ and P-cells; the latter are not driven by central I activity. Previous work from this laboratory (Averill et al., J. Neurophysiol. 52:771-785, 1984) has shown, using single unit cross-correlation analysis, that Iβ and P-cells are monosynaptically excited by PSRs. Further, connectivity from any one PSR was seen in 20% of cases for Iβ neurons and 26% of cases for P-cells. But cross-correlation analysis may not be able to detect PSR-evoked unitary EPSPs of small amplitudes (Cope et al., Soc. Neurosci. Abstr. 8: 448, 1982). In contrast, Backman et al. (Pflügers Arch. 402:129-136, 1984), using intracellular STA, observed PSR-derived monosynaptic EPSPs in all cells they classified as Iβ (N=11 cells), that is, a 100% connectivity. This connectivity is surprisingly high and may be a consequence of their classifying a neuron as Iβ if it received an observable volley of EPSPs in response to lung inflation.

To investigate PSR connectivity, we used intracellular STA on a large population of DRG neurons in anesthetized, paralyzed, artificially ventilated cats. Single-unit PSR activity was recorded in the nodose ganglion and intracellular membrane potentials were recorded from neurons in the ipsilateral DRG. We classified DRG neurons based on a comparison of cycle-triggered histograms (CTH) of intracellularly recorded membrane potentials and action potentials, using an inflate and no-inflate paradigm (Cohen and Feldman 36:2367-2374, 1977). We studied the connectivity of only those PSRs exhibiting extracellularly recorded terminal potentials in the DRG (Berger and Averill, J. Neurophysiol. 49:819-830, 1983).

Fifty intracellularly recorded DRG neurons were classified as either Iα, Iβ or P-cells. Twelve of 25 Iβ neurons showed evidence of a PSR-derived unitary monosynaptic EPSP (mean amplitude 70 μV, range 15-254 μV). Two of four P-cells exhibited EPSPs (amplitudes of 120 and 239 μV). No unitary EPSPs were observed in the 21 Iα neurons investigated. We estimate that a minimal detectable EPSP was 5-10 μV in amplitude. We conclude that the percentage connectivity of a single PSR to an Iβ neuron is approximately 50% for neurons classified on the basis of CTH analysis of their responses to lung inflation. In a large sample of Iα neurons, we observed no evidence for PSR-derived EPSPs. The absence of this connectivity is consistent with a lung-volume-derived reduced excitability of Iα activity. (Supported by USPHS grant NS 14857, and a Parker B. Francis Fellowship to T.E.D.)

339.1 THE INFLUENCE OF AGE ON AUGUST LEVELS OF PINEAL IMMUNOREACTIVE ARGININE VASOTOCIN (iAVT) IN RATS. M.M. Prechel*, T.K. Audhya* and W.H. Simmons* (SPON: F.W. Lavelle). Dept. of Biochemistry and Biophysics. Loyola Univ. Med. Ctr., Maywood, IL 60153.

The level of iAVT in rat pineal glands varies with a circannual rhythm; it is barely detectable during most of the year but increases several hundred-fold in August (Prechel et al., Endocrinology 112: 1474, (1983)). It has been reported that AVT bioactivity in pineal diminishes rapidly with age (Pavel et al., J. Endocrinol. 66: 283, (1978)). However, we have observed that the August iAVT peak is qualitatively and quantitatively similar in both weanling and adult rats (Prechel et al. , Endocrinology 112: 1474, (1983)); J. Pineal Res. 1: 175 (1984)). The purpose of this study was to systematically evaluate the influence of age on pineal iAVT levels during August when the hormone is readily measurable.

Pineal glands were collected from separate groups (n=4) of either male or female rats (Sasco-King, Oregon WI) at 14, 34, 54, and 74 days of age on August 4, 12 and 19, 1984. The glands, obtained after decapitation, were immediately frozen on dry ice and then stored at -80°C. Glands were later homogenized in 0.1N acetic acid containing $10^{-6}$M pepstatin and subsequently heat denatured to precipitate large proteins. The supernatants from individual glands were assayed for iAVT by RIA (Fernstrom et al., Endocrinology 106: 243, (1980)). The data were subjected to a three-way ANOVA to determine the influence of age, gender and sampling date on iAVT.

The results of this study confirm that the level of pineal iAVT varies in a highly significant way with date of sampling during August. However, all groups of rats reached the same peak-level of pineal iAVT during August (700-750 pg/gland) regardless of age or gender. Minor, but significant, interactions were observed between age and sampling date, as well as between gender and sampling date, suggesting that age and gender may influence slightly the phasing of the annual peak of iAVT.

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339.2 ISOLATION OF cDNA CLONES FOR RETINAL S-ANTIGEN. Cheryl M. Craft[1], Paul Stein[2], David C. Klein[1], Igal Gery [2] and Toshimichi Shinohara[3]
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S-antigen is a soluble protein (50 KD) which is originally isolated from bovine retinal photoreceptor cells. Recent immunological studies indicate it is present in all animal photoreceptor cells and pineal glands. This protein is of special interest because it is capable of inducing experimental uveitis in animals and is thought to be involved in the process of visual transduction. Complementary DNA (cDNA) clones for bovine retinal S-antigen were isolated from a λgtll recombinant DNA expression library by polyclonal S-antigen antiserum. These clones were further verified by monoclonal antibodies. Subsequent cDNA clones from a λgtl0 retinal library were also screened using the 500 base pair cDNA from the λgtll library as a probe. Several S-antigen cDNA clones with approximately 250 to 900 base pair inserts were analyzed using restriction enzymes. Polypeptide synthesized with these cDNA clones greater than 400 base pairs has at least 2 different epitopes recognized by 2 different monoclonal antibodies. In contrast, smaller than 400 base pairs have at least one epitope. These polypeptides were unable to induce uveitis in the experimental animals indicating that these cDNA clones may not encode the uveitogenic determinant. A 500 base pair S-antigen cDNA was found to hybridize specifically to mRNA (approximately 1700 ± 200 nt) prepared from bovine retina and pineal gland but not to mRNA from liver, cerebral cortex or cerebellum. This is consistent with SDS-PAGE immunological analysis, which indicates that the retina and pineal gland contain the S-antigen. These S-antigen cDNA clones will be a useful tool in future studies of the S-antigen gene.

339.3 MODIFICATIONS OF SECRETORY PATTERNS OF PINEAL MELATONIN IN THE RABBIT. Y.M. Cheung*, Y.S. Chan and S.F. Pang*. Department of Physiology, Faculty of Medicine, University of Hong Kong, Sassoon Road, Hong Kong.

In rodents, pineal melatonin is secreted into the confluens sinuum before entering the general circulation. Investigation on blood levels of melatonin in the confluens sinuum provides direct measurements on the secretory patterns of pineal melatonin in these animals. In our laboratory the sinus sagittalis superior of the anaesthetized rabbit (Sagatal, May & Baker, 35 mg/kg i.v.) was cannulated and blood samples from the confluens sinuum were collected at 2- or 4-minute intervals. Plasma melatonin were extracted by dichloromethane and quantified by radioimmunoassay. It was found that the confluens sinuum had melatonin levels 4-20 times higher than those in the peripheral circulation. In addition, melatonin levels in the sinus blood exhibited episodal release pattern and diurnal variation with high levels in the dark period. When the sympathetic trunk innervating the pineal gland was stimulated unilaterally by electrical pulses (0.5 ms pulses, 300 Hz, continuous pulse trains usually lasting 8 s repeated once every 20-25 s), melatonin levels in the confluens sinuum increased to over 130% of the prestimulated values. However, unilateral electrical stimulation of the sympathetic nerve by pulse trains of shorter duration (0.5 ms pulses, 300 Hz, pulse trains of 60 ms duration repeated once every 500 ms) had no apparent effect. Injection of norepinephrine which caused a transient increase in blood pressure also enhanced concentrations of plasma melatonin in the confluens sinuum by over 50%. Our data suggest that the secretion of pineal melatonin in the rabbit has: 1) a pulsatile release pattern superimposed on a basal level of secretion, 2) a diel rhythm, and 3) a regulatory system that is stimulated by circulating norepinephrine and activation of its sympathetic innervation.

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339.4 REPRODUCTIVE EFFECT OF O-ACETYL-5-METHOXYTRYPTOPHOL (aML) IN MALE SYRIAN HAMSTERS. M.K. Vaughan, B.N. Joshi*, M.E. Troiani*, G.M. Vaughan*, I. Smith* and R.J.Reiter. Dept. Cellular & Structural Biology, Univ. Texas Health Science Center, San Antonio, TX 78284; U.S. Army Institute of Surgical Research, Fort Sam Houston,TX 78234 and Courtauld Institute of Biochemistry, London, U.K.

O-acetyl-5-methoxytryptophol (aML), an indole with only an O substituted for the sidechain N of melatonin (aMT), has been identified in the pineal gland (Smith et al., Biochem. J. 185: 537, 1980). This new pineal indole was tested for its ability to: 1) cause gonadal regression like aMT when aML is injected each afternoon into hamsters exposed to a 14:10 light:dark (LD) cycle and 2) prevent gonadal regression like aMT when implanted as a chronically releasing subcutaneous pellet in hamsters exposed to short photoperiod (SP, 10:14 LD) or injected each evening with aMT. In Exp. 1, hamsters received either saline injections (controls) or 2.5, 12.5, 25 or 50 µg/day at 1700 h of synthetic aML. Testes weights were not significantly depressed by any dose of aML although accessory organ weights were depressed by the 50 µg dose. In Exp. 2, hamsters in SP or receiving afternoon injections of aMT were implanted every 2 weeks for 12 weeks with a beeswax pellet containing 1 mg aML. Subcutaneous pellets of aML did not prevent the testicular or accessory organ weight regression induced by exposure of hamsters to SP or daily afternoon injections of aMT. The results indicate that aML does not have the same antigonadotrophic properties that aMT has in the Syrian hamster (Supported by NSF grant No. PCM8410592).

339.5 STRESS DEPRESSES N-ACETYLTRANSFERASE ACTIVITY (NAT) AND MELATONIN LEVELS IN THE PINEAL GLAND OF THE RAT. M.E. Troiani*, B.N. Joshi*, J. Milin*, F. Nürnburger*, and R.J. Reiter. Dept. Cellular and Structural Biology, Univ. Texas Hlth. Sci. Ctr. at San Antonio, TX 78284.

It was recently shown that a 1.5 cc injection of physiological saline into the back leg of rats caused morphological changes in the pineal gland which were interpreted as evidence that the secretory activity of the pineal gland was increased (Milin, J., et al., Arch. d'Anat. Micro., 73: 159, 1984). This observation prompted the present series of studies which examined pineal NAT activity and melatonin content after animals were stressed in the manner described. In the initial study, young adult male rats were kept under light:dark cycles of 14:10 (lights on from 0600-2000 h) and given a saline injection into the back leg during the light phase at 18:30 h. Ten minutes later the already low daytime levels of melatonin were significantly depressed. Because of this unexpected finding a time course study was conducted during darkness when pineal NAT activity and melatonin levels were increasing. At 0200 h the experimental rats received a subcutaneous saline injection as described above; these were killed at either 10, 20, 30, 40, 60 or 90 min after the injection. Control animals remained untreated and were killed at either 0 or 30 min. Pineal NAT activity roughly doubled in the control rats between 0200-0230 h. In contrast, pineal NAT values dropped precipitously to roughly 1/5 the initial control values within 20 mins; NAT levels remained significantly depressed until 60 min after the saline injections. By 90 min the NAT values were returned to those in the controls. Melatonin levels responded similarly. In control rats, pineal melatonin levels rose from 1712 at 0200 h to 3322 pg/gland at 0230. In contrast pineal saline-injected rats contained only 1332 pg/gland at 0230 h; however, as with the NAT, melatonin levels had returned to control values at 90 min after the saline injection. In the next study, the nighttime response of the pineal to the stress was compared in intact and adrenalectomized rats. Adrenalectomy totally prevented the changes in NAT activity associated with the saline injection. In the case of melatonin, the absence of the adrenals not only prevented the drop in melatonin but these animals had higher melatonin values, compared to those in controls, at 20 min after the injection. It is presumed that the observed alterations are due to changes in adrenomedullary catecholamines rather than to adrenocortical gluco- or mineralocorticoids. These findings seem to be opposed to the general dogma that increased circulating catecholamines normally would exaggerate pineal melatonin production. Supported by NSF grant # PCM 8410592.

339.6 SIMILAR EFFECTS OF SHORT PHOTOPERIOD AND MELATONIN TREATMENT ON GONADOTROPINS IN OVARIECTOMIZED INBRED LSH/SsLak HAMSTERS. U. E. Hauser* and B. Benson. Dept. of Anatomy, Univ. of Arizona, Tucson, AZ 85724.

Previous experiments carried out in inbred LSH/SsLak female hamsters showed that these animals become acyclic after 20 to 30 days of short photoperiod (SP) exposure, and have reduced postcastration gonadotropin rises compared with long photoperiod (LP) exposed animals. Since melatonin (MEL) is synthesized in the pineal gland and is postulated to be the hormone mediating SP effects on the reproductive system, the following experiment was designed to compare directly gonadotropin levels in SP and MEL treated animals.

Forty 4-5 month old, regularly cycling LSH/SsLak female hamsters were maintained in LP (14L:10D cycle; lights on at 6.00 h). On the day following bilateral ovariectomy, the animals were divided into five groups, two of which were transferred to SP (8L:16D cycle; lights on at 6.00 h). One group in each photoperiod received daily subcutaneous injections of either 25 µg MEL or the vehicle; one LP-exposed group remained untreated. Daily injections were given between 15.30 and 16.00 h in LP animals and 13.30 and 14.00 h in SP animals. After 20 days of treatment all animals were sacrificed during morning hours and sera and pituitaries saved for hormonal determinations. NIADDK kits with LH-RP-2 and FSH-RP-2 standards were used for hormonal determinations by RIA.

| | LH | | FSH | |
|---|---|---|---|---|
| Treatment | Serum(ng/ml) | Pit.(µg) | Serum(ng/ml) | Pit.(µg) |
| LP-Control | 0.90±0.20 | 1.80±0.13 | 40.3±4.5 | 0.91±0.06 |
| LP-MEL | $^{b}$0.34±0.02 | 1.51±0.12 | $^{c}$14.0±1.1 | $^{c}$0.65±0.05 |
| SP-Vehicle | $^{b}$0.37±0.03 | $^{b}$1.30±0.38 | $^{c}$20.8±2.4 | $^{a}$0.69±0.07 |
| SP-MEL | $^{b}$0.38±0.07 | 1.55±0.13 | $^{a}$14.9±1.4 | $^{b}$0.68±0.06 |

$^{a}p < 0.05$; $^{b}p < 0.025$; $^{c}p < 0.005$. All vs. LP-Control.

The results are summarized above. Since values in LP vehicle-treated and untreated groups were not significantly different, they were pooled. Serum and pituitary FSH levels were depressed by either SP or MEL treatment. MEL given to SP animals did not produce an additive effect. In response to SP or MEL, LH levels showed similar trends. These results indicate that SP and MEL treatments have comparable suppressive effects on gonadotropin levels in the absence of gonadal steroids, and also provide further support for the hypothesis that MEL may mediate the antigonadal effects of SP.

This work was partially supported by N.I.H. grant no. HD18570.

339.7 THE EFFECT OF ELECTRICAL STIMULATION OF THE PARAVENTRICULAR NUCLEUS AREA OF THE HYPOTHALAMUS ON THE MELATONIN RHYTHM GENERATING SYSTEM OF THE RAT. J. Yanovski*, J. Witcher*, N. Adler, S. Markey* and D. Klein*. Univ. of Penn. Dept. of Psychology, Phila., PA and National Institutes of Health, Bethesda, MA.

The circadian rhythm in mammalian pineal melatonin production appears to be controlled by the Suprachiasmatic Nuclei via a polysynaptic pathway. Previous studies have suggested that the Paraventricular Nuclei (PVNs) may be intermediate in this pathway; the PVNs receive efferents from the Suprachiasmatic Nuclei and send efferents to the spinal cord (and thus to the Superior Cervical Ganglion, the final common pathway for pineal neural input). Additionally, lesions of the PVNs have been shown to eliminate the circadian rhythm in pineal melatonin production as measured in a variety of ways (Klein et al, 1983).

The present study seeks a role for the PVNs in the melatonin rhythm generating system by examining the effects of electrical stimulation of the PVNs on levels of urinary 6-OH-Melatonin, the major metabolite of Melatonin. Female Sprague Dawley CD rats, implanted with unipolar electrodes, were placed in metabolic cages under a 14:10 light/dark cycle and their urine collected over dry ice. Each animal's urine was collected as day time and night time specimens for three day periods consisting of: 1) a 24 hour control period; 2) a 24 hour period in which 3 hours of electrical stimulation of the Paraventricular Nucleus area began either one hour after lights-on or one hour after lights-off; and 3) a second 24 hour control period following the stimulation period. The urine of all animals was collected under both types of electrical stimulation conditions, with a minimum of two weeks between stimulations.

Histological verification of electrode placement was obtained. All urine samples were evaluated for 6-OH-Melatonin content by Gas Chromatography-Negative Chemical Ionization Mass Spectroscopy (Tetsuo et al, 1980).

In animals with electrode placements in the PVNs, electrical stimulation during the early light induced a three to four fold rise in the lights-on urinary 6-OH-Melatonin concentration as compared to that produced on control days. Stimulation during the early dark in these same animals did not uniformly induce a significant rise in urinary 6-OH-Melatonin levels.

These results are consistent with a role for the PVNs in the polysynaptic circuit controlling the melatonin circadian rhythm generating apparatus. A more fine-grained examination of the effects of electrical stimulation at differing times of day and night is currently under way.

339.8 DAILY ISOPROTERONOL INJECTIONS INDUCE GONADAL REGRESSION IN ADULT MALE DJUNGARIAN HAMSTERS. T.H. Champney and M.H. Stetson. School of Life and Health Sciences, University of Delaware, Newark, DE 19716.

When exposed to short photoperiods (less than 14 hours of light per day), male Djungarian hamsters (Phodopus sungorus sungorus) exhibit marked gonadal regression and increased duration of pineal melatonin production during the dark period. Likewise, daily melatonin injections during the late afternoon can also elicit gonadal regression in this species; ostensibly by adding to the endogenous melatonin produced and thereby simulating a long night (increased duration of melatonin exposure). Isoproterenol, a potent beta adrenergic agonist, stimulates pineal melatonin production in Djungarian hamsters. Therefore, the purpose of this study was to determine if endogenous melatonin release, induced by isoproterenol injection, could produce the same alterations in the reproductive state as those observed in animals which are exposed to short photoperiods or injected with melatonin.

Male Djungarian hamsters exposed to LD 16:8 (lights on at 0500) were divided into three groups and received daily subcutaneous isoproterenol injections (0.75 mg/kg) at either 1000, 1600, or 1800 hours. After eight weeks of injections, the hamsters were killed and their body and testes weights were recorded. No significant differences were observed in body weight between any of the groups. However, testes weights were significantly depressed in the groups injected at 1600 ($p < 0.05$, $n = 9$) and 1800 ($p < 0.001$, $n = 7$) hours, while the testes weights of the animals injected at 1000 ($n = 12$) hours were unchanged by the isoproterenol treatment. All of the hamsters injected at 1800 hours had completely regressed testes, while only 2 of 9 animals at 1600 hours showed complete regression after eight weeks of injections. These results are very similar to those in hamsters which receive melatonin injections at these same times.

These findings demonstrate that stimulating production of pineal melatonin, at a specific time of day, causes gonadal regression in male Djungarian hamsters. Supported by NSF Research Grant DCB 84-12587.

340.1 BIOCHEMICAL AND BEHAVIORAL CORRECTION OF MPTP PARKINSON-LIKE SYNDROME BY FETAL CELL TRANSPLANTATION, R.A.E. Bakay,* D.L. Barrow,* A. Schiff* and M. Fiandaca* (SPON:J.W. Manning) Emory University, Atlanta, Ga. 30322.

We have successfully produced a Parkinsonism-like syndrome using MPTP in non-human primates and have reversed the syndrome through transplantation of fetal mesencephalic brain tissue. Movement was both qualitatively and quantitatively assessed through videotape recordings which were scored by a blinded observer. Following the administration of MPTP the overall quality of the movements decreased, the movement repertoire decreased and the time spent in certain types of movements significantly stastistically diminished as much as 50%. Sedimentary behavior appeared to increase and there was a stastistically significant increase in the time spent in one particular type of movement without change to a different type of movement pattern. Corresponding with these movement changes, there was a decrease in CSF L-Dopa representing as much as an 80% reduction.

Two Macaca Mulattas received fetal mesencephalon cell preparations stereotactically implanted in multiple sites of the caudate nucleus bilaterally. These animals demonstrated qualitative improvements in their movements and stastistically significant quantitative increases in the amount of active behavior observed over a two month period. Despite these improvements the repertoire remained somewhat limited. CSF L-Dopa increased to baseline concentrations.

Neuropathological studies have been performed which demonstrate the successful integration of fetal tissue cells in the caudate. Catecholamine-fluorescent cells were observed in the transplant. No evidence of rejection was present. We therefore feel that fetal tissue transplantation in primates can effectively reverse behavioral abnormalities and biochemical abnormalities induced by MPTP. Long term studies are being preformed to determine whether or not the transplant can survive and function chronically.

340.2 BEHAVIORAL, BIOCHEMICAL AND PATHOLOGICAL CHARACTERISTICS OF AN MPTP MODEL OF PARKINSON'S DISEASE IN THE CAT. J.S. Schneider, A. Yuwiler and C.H. Markham. Department of Neurology, UCLA School of Medicine and Neurobiochemistry Lab; Brentwood V.A. Hospital, Los Angeles, CA 90024.

Systemic injections of MPTP (N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) cause a parkinsonian syndrome in humans and non-human primates. Severe loss of pigmented substantia nigra (SN) compacta cells has been documented in both cases. While transient dopamine depletions have been observed in other species exposed to MPTP (rat, guinea pig) structural changes in the SN have not been well documented. We presently report that MPTP administered to cats can produce severe SN compacta cell damage, long-lasting striatal dopamine (DA) depletions and a behavioral syndrome with many similarities to that observed in both humans and non-human primates.

Following administration of MPTP (5-10 mg/kg, i.p., 5-7 days) cats quickly exhibit drooling, shaking, pupil dilation, apparent hallucinations (cats adopt attack postures, hiss violently, track imaginary objects, pinnae in constant motion), and become hypersensitive to sensory stimulation.

Subacutely, pronounced motor deficits are observed. Cats exhibit hypo- and bradykinesia, loss of or severe impairment of postural reflexes, drooling, reduced response to sensory stimuli, decreased vocalization, flexed, abnormal postures, and "freezing" episodes during attempted movement. Abnormal postures and hypo- and bradykinesia are reversed with L-dopa treatment. The degree of motor impairment following a given dose of MPTP is somewhat variable (from very mild to very severe) and dependent to some extent upon the age of the animal. To date, all cats (both those mildly and severely affected) have recovered gross motor functions 3-4 weeks after MPTP administration.

Severely affected animals had at least an 85-90% striatal DA depletion and severe SN compacta cell loss and associated glial proliferation. A few remaining SN cells looked relatively normal but many more appeared pyknotic. Mildly affected animals had 30% decrease in striatal DA and many normal-looking SN compacta cells. Behavioral recovery (at one month) did not coincide with recovery of striatal DA content.

The recovery of function observed in these animals may be due to functional recovery of noradrenergic and serotonergic neurons, compensatory increases in surviving SN cell DA synthesis,increased transmitter release from DA terminals, DA receptor proliferation, or some combination of these factors. Studies in the cat should provide a good means for assessing the biochemical and physiological consequences of MPTP exposure.

340.3 GLOBUS PALLIDUS UNIT ACTIVITY IN THE MONKEY DURING THE INDUCTION OF PARKINSONISM BY 1-METHYL-4-PHENYL-1,2,3,6,-TETRAHYDROPYRIDINE (MPTP). M. Filion, R. Boucher and P. Bédard, Lab. Neurobiol., Enfant-Jesus Hosp. and Laval Univ., Quebec, Canada G1K 7P4.

We have already described abnormal neuronal activities in the globus pallidus of monkeys made parkinsonian by electrolytic midbrain lesions (Brain Res., 178 (1979) 425-441). These anomalies were related to the amount of cell loss in the pars compacta of the substantia nigra. Recently, the neurotoxin MPTP was shown to destroy these nigral neurons much more specifically and to produce permanent parkinsonism in primates. The present work was undertaken to determine whether MPTP also induces alterations of pallidal activities similar to those reported above.

We recorded the spontaneous activity of over 150 neurons in the internal (GPi) and external (GPe) segments of the globus pallidus. Recordings were made daily during one week before and 5 weeks after the i.v. injection of MPTP (0.6 mg/kg). The animal gradually developed severe bradykinesia, apraxia, and tremor. These signs remained present until he was sacrificed. However, some recuperation occurred. Thus, on day 14 the animal started to regain the ability to feed himself, lost completely on day 4.

During the first 2 weeks after MPTP, the tonic firing pattern characteristic of GPi neurons, and the long bursts and long periods of silence, typical of GPe, both became more and more disorganized. Rates of firing were measured over periods of minutes. Unexpectedly in GPi, they were on average about 50% higher than those recorded nearby in the same segment of the nucleus before MPTP. Inversely in GPe, during the same period, firing rates were about 50% lower.

After the second week the abnormal activities were clearly similar to those observed in parkinsonism due to electrolytic lesions. Short irregular bursts were present in both GPe and GPi. Rhythmic bursting at 15 Hz, often observed in monkeys with electrolytic lesions, was first recorded on day 14, and then appeared intermittently in more and more neurons. After day 14, firing rates were close to normal in GPi, as in monkeys with electrolytic lesions; they remained somewhat slower in GPe.

In conclusion, MPTP induces alterations of pallidal activities similar to those observed in parkinsonism due to electrolytic lesions. This becomes obvious, however, only several days after the injection of the neurotoxin. Early effects of MPTP, such as changes in firing rates, were probably present in the animals with electrolytic lesions but were overlooked since most of these animals were studied several weeks after making the lesions. Nevertheless, the fact that the early effects of MPTP appear to be somehow compensated suggests a functional reorganization that warrants further studies. [Supported by the MRC of Canada].

340.4 Persistent Alterations in locomotor activity of Mice following treatment with MPTP. J.L. Carlson*, M.E. Melnick and L.L. Vacca. Department of Anatomy and Department of Physical Therapy Education, University of Kansas Medical Center, Kansas City, Kansas 66103.

One of the criteria for a rodent model of Parkinson's Disease utilizing MPTP is that the motoric effects of the drug persist beyond the injection period. Previous results in this laboratory (Shellenberger & Melnick, Neurosci Abstr 1984) have shown that alterations in activity levels and locomotion persist at least 10 days after injection with MPTP in one year-old rats. This study investigated long term changes in activity and locomotion in $C_{57}$ mice. The mice were injected for 3 days, 5 days or 10 days with either MPTP at 30 mg/kg or a control solution. Activity levels were investigated immediately after the first injection and then at 5 day intervals up to 33 days after injections had ceased by measuring movement in a continuous maze. Locomotion was assessed on the same schedule by gait analysis measuring stride length, stride width and the ratio of width to length. Activity was severely depressed one hour after injection; was increased in the three-day injection group and slightly depressed in the five-day and ten-day injected group at 5, 10, and 15 days when compared to controls. Activity levels were then within normal limits. Gait analysis showed that the treated animals took shorter, wider steps within one hour of injection. These gait alterations persisted for thirty days for all treatment paradigms. We conclude that there is long-term impairment in motoric activity of $C_{57}$ mice after injections with MPTP. The brain of these animals are now being analyzed to see if there are accompanying changes in immunohistochemical staining for tyrosine hydroxylase and/or changes in the number of perikarya in the Substantia Nigra.

340.5 EFFECTS OF MPTP ON BASAL GANGLIA PHYSIOLOGY IN MONKEYS. E.B. Montgomery, Jr., S.R. Buchholz*, A. Delitto*, and R.C. Collins. Dept. of Neurol. and Neurol. Surg. (Neurology) and Program in Phys. Ther., Washington Uni. Sch. of Med., St. Louis, MO 63110

Effects of MPTP on the physiology of the basal ganglia (BG) in two macaca nemestrina monkeys were studied combining extracellular neural recording of single unit activity and 14-C-2-deoxyglucose autoradiography. Mean resting discharge frequencies in spikes/sec (MRDF) for 41 units in the putamen and 56 units in the globus pallidus compared to 40 units in the putamen and 53 units in the globus pallidus following total cumulative MPTP doses of 2.7 mg/kg for monkey A and 1.4 mg/kg for monkey B. Prior to sacrifice each monkey was given 14-C-2-deoxyglucose (2-DG) under conditions similar to those during single unit recording.

Values for MRDF for putamen (median MRDF 3.7, range 0.35-40) and for globus pallidus (median 31, range 1.7-122.2) before MPTP were similar to those reported by Crutcher and DeLong (Exp. Brain Res. 53:244-258, 1984). Values for MRDF following MPTP were not significantly changed (Kolmogorov-Smirnov test). 2-DG results were: (micromoles of glucose per 100 gms of tissue per minute)

| | normal | | | parkinsonian | |
|---|---|---|---|---|---|
| | A | B | C | A | B |
| caudate nucleus | 72 | 52 | 70 | 77 | 88 |
| putamen | 68 | 53 | 70 | 83 | 95 |
| globus pallidus external segment | 22 | 26 | 38 | 60.5 | 69 |
| globus pallidus internal segment | 24 | -- | -- | 56.5 | 68 |

Normal values reported in the literature: A.) Caveness, Ann. Neurol. 7:230-237, 1980; B.) Kennedy, Ann. Neurol. 12:333-340, 1982; and C.) Deuel and Collins, Ann. Neurol. 15:521-529; 1984.

Our findings of mildly increased glucose utilization in the putamen and a 2-fold increase in the globus pallidus are in agreement with changes in rats following unilateral 6-hydroxydopamine lesions of the substantia nigra (J. Neurosci. 1:285-291, 1981) and in PET scans of human parkinsonians (Canad. J. Neurol. Sci. 11:169-173, 1984). Yet, single unit activity in "parkinsonian" monkeys at rest was normal. However, the experimental conditions for the single unit recording and 2-DG were not strictly the same. In single unit recording any sample collection contaminated by movement was rejected. This is not feasible in 2-DG experiments because of the 50 minute sampling period during which the monkeys invariably move. Transient increases in glucose utilization associated with occasional movements are additive because phosphorylated 2-DG is essentially trapped intracellularly. It is possible that the increased BG 2-DG uptake following MPTP reflects changes from normal glucose utilization associated with movements occurring during the 2-DG experiment. If so, then the pathophysiology of parkinsonism is related to abnormal dynamic changes in neuronal activity associated with movement production and not in the resting activity.

340.6 INTRACELLULAR RESPONSES IN THE CAUDATE NUCLEUS OF A PARKINSONIAN DOG. J.S. Wilson & J.A. Wilson. Department of Anatomy, Howard University, Washington, D.C. 20059 & Lab. of Preclinical Studies, Nat. Inst. on Alcohol Abuse & Alcoholism, Rockville, M.D. 20852.

Systemic injections of MPTP (methyl-4-phenyl-1,2,3,6-tetrahydropyridine) induce a selective degeneration of the pars compacta of the substantia nigra, depletion of striatal dopamine levels and a Parkinsonian-like syndrome in man, monkey, dog and mouse. Because degeneration of the nigrostriatal pathway is often associated with Parkinson's disease, it is thought that the substantia nigra may modulate inputs to the striatum which are directly related to motor control such as cortical area 4. The purpose of this research is to study the changes which occur in the physiology of the caudate (Cd) nucleus in dogs which have developed a Parkinsonian-like syndrome because of systemic injections of MPTP.

Recordings were made in the head of the Cd nucleus of normal beagle puppies and puppies which were injected with MPTP (2.5-3.0 mg/kg; i.v.) 12-18 days before the recording experiments. Stimulating electrodes were placed in the primary motor cortex, supplementary motor cortex and premotor cortex. Preliminary results are based on 21 and 15 neurons recorded intracellularly in the normal and MPTP treated dog, respectively. In the normal dog, 16 of the neurons responded to at least one of the 3 cortical stimulation sites. The most frequently recorded response was an initial excitation followed by a longer duration inhibition, ie. an E-I response complex. Usually, one of the three stimulation sites was more effective in producing an E-I response. In the MPTP treated dog, cortical stimulation produced a response in 13 neurons. E-I responses were observed in 5 of the responding cells. In the remaining 8 neurons, the responses were unusual in that the initial excitatory response often had a larger amplitude and longer duration than was observed in control conditions. In addition, the inhibitory component of the response was greatly attenuated or absent. Our preliminary data also suggest that Cd neurons can follow repetitive stimuli at higher frequencies in the MPTP treated dog. We are continuing this study to further document the changes in the response of Cd neurons to cortical inputs in the Parkinsonian dog. (Supported by MBRS 2S06-RR08016-13).

340.7 ACUTE EFFECTS OF MPTP ON THE FIRING RATE OF NIGRAL PARS COMPACTA CELLS IN RATS AND MICE. H. Klemfuss*, R.F. Gariano, and P.M. Groves.* Department of Psychiatry, School of Medicine, University of California, San Diego, La Jolla, CA 92093.

Neurons in the pars compacta of the substantia nigra were recorded extracellularly in male Sprague-Dawley rats and C57 Black mice anesthetized with urethane. Based on waveform, spontaneous firing rate, and antidromic activation from the striatum, the cells were considered to be dopaminergic nigrostriatal projection neurons. MPTP (1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine) base was dissolved in either 1% ethanol or normal saline and administered subcutaneously.

In the rat 10 mg/kg MPTP resulted in a 10-20% decrease in the spontaneous firing rate of pars compacta cells which developed within five minutes and lasted at least thirty minutes ($p<.05$ at ten minutes post-injection, n=5). Mice were somewhat more sensitive to the rate-depressant effects, with a decrease to 49% of pre-injection firing rate at ten minutes ($p<.01$, n=5). At a dose of 30 mg/kg MPTP there was a similar decrease in the firing rate of mouse pars compacta cells to 50% at ten minutes post-injection ($p<.01$, n=6), although this dose was ultimately lethal. Injection of vehicle alone resulted in no significant effect on the firing rate of these cells ($p>.10$, n=9).

Our results show that after acute administration, MPTP has a rate-depressant effect on nigrostriatal dopamine cells in rodents. This evidence argues that the action of MPTP can be distinguished from neurotoxic agents which cause a hyperexcitation of neuronal activity immediately after acute administration. Preliminary work using the method of terminal excitability testing indicates that the acute effects of MPTP can also be discriminated from dopamine-releasing agents such as amphetamine. We are studying the actions of MPTP on terminal excitability in the rat and mouse.

340.8 MPTP AND MPDP+ CAUSE A NON-REVERSIBLE DEPRESSION OF SYNAPTIC TRANSMISSION IN MOUSE NEO-STRIATAL BRAIN SLICE. J.A. Wilson, F.F. Weight, & J.S. Wilson Lab. of Preclinical Studies, Nat. Inst. on Alcohol Abuse & Alcoholism, Rockville, MD 20852. & Dept. of Anatomy, Sch. of Med., Howard Univ., Washington, DC 20059.

MPTP (1-methyl, 4-phenyl, 1,2,3,6,tetrahydropyridine) causes a Parkinson's disease-like syndrome in man, monkeys, dogs and mice (Lewin, Science 225: 1460, 1984). The mechanism of MPTP's neurotoxicity is not known; however, it has been found that the toxic effects are prevented by either dopamine uptake blockers or by monoamine oxidase inhibitors (MAO) (Heikkila et. al., Nature 311: 467, 1984). Two compounds, MPDP+ and MPP+, have been identified as products of MPTP's oxidation. We studied the effects of these compounds, as well as MPTP, on synaptic transmission in neo-striatum using a brain slice from C57 black mice. The preparation, stimulation, and recording parameters were similar to those reported by Cordingley & Weight (Soc. Neurosci. Abs. 8: 373, 1982) for the rat neo-striatal slice. The neo-striatum was stimulated using bipolar electrodes and a two component field was recorded extracellularly; the first component, N-1, is a negative going fiber volley, and the second component, N-2, is a synaptic response. MPTP caused a decrease in the amplitude of N-2 (62 $\pm$ 1.4% of control, n=9) that was not reversed with up to 4 hours of wash. This non-reversible change was blocked by the addition of either pargyline, a MAO inhibitor or GBR-32, a selective dopamine uptake blocker, to the slice prior to and during MPTP application. Thus compounds which block the effects of MPTP in vivo also block the non-reversible decrease in N-2. MPP+ also caused a change in N-2; however, the change in amplitude was reversible and returned to the control value within a 2 hour wash period (107 $\pm$ 1.3% of control, n=8). Thus, the presence of MPP+ in the slice does not appear to be responsible for the non-reversible actions of MPTP. MPDP+, like MPTP, caused a non-reversible decrease in the amplitude of N-2 (46 $\pm$ 5.8% of control, n=4). Castagnoli et. al. (Life Sci. 36: 225, 1985) have proposed that MPDP+ may oxidize dopamine to toxic quinones while being reduced to MPTP. MAO would then reoxidize MPTP to MPDP+ and the cycle could continue. If this proposed cycle is responsible for forming toxic compounds, pargyline should decrease or prevent MPDP+'s toxicity. Application of pargyline prior to and simultaneously with MPDP+ produced a reversible decrease in N-2 amplitude which returned to control amplitude in less than 1 hr. of wash (110 $\pm$ 7.4% of control, n=3). These results suggest that the cyclical reduction of MPDP+ and oxidation of MPTP are crucial for the non-reversible depression of synaptic transmission we have observed in the mouse neo-striatum.

We thank Dr. W. Gessner for the gift of the MPDP+. J.A.W.'s current address: Dept. of Physiology, School of Medicine, Creighton Univ. Omaha, NE 68178.

340.9 NEURONAL ACTIVITY IN AREA 4 RECORDED IN TRAINED MONKEY AFTER I.V. ADMINISTRATION OF 1-METHYL-4-PHENYL, 1,2,3,6-TETRAHYDROPYRIDINE (MPTP). D. Doudet*, Ch. Gross*, Ph. Lebrun-Grandié* and B. Bioulac, Lab. of Neurophysiology, Groupe Motricité, Univ. of Bordeaux II, 33076 BORDEAUX CEDEX (FRANCE).

Numerous reports (Burns et al., Proc. Natl. Sci. USA, 80 : 4546, 1983 ; Langston et al., Brain Res. 292 : 390, 1984 ; Doudet et al., Brain Res., sous presse, 1985). Showed that the systemic administration of 1-Methyl-4-Phenyl, 1,2,3,6, tetrahydropyridine (MPTP) in monkeys induces (1) hypertonia, hypokinesia and similar perturbations in movement parameters and EMG activity as observed in Parkinson's disease and (2) a selective degeneration of the dopaminergic neurons located in the pars compacta of the substantia nigra. Although it exists many data concerning the modulatory influence exerted by the sensory feed back loop (Bioulac and Lamarre, Brain Res., 172 : 427, 1979) and the cerebellum (Lamarre et al., J. Physiol. (Paris) ; 74 : 253, 1978) on the neuronal activity of the motor cortex (area 4), there is very little data concerning the role of the SN in this modulation (Gross et al., Exp. Brain Res., Suppl. 7, 181 : 1983). By using the MPTP primate model for the lesionning of the SN, the aim of the present work was to appreciate its possible influence on the unit activity of area 4. **Methods**. Extracellular unit activity of motor cortex was recorded in two monkeys (Macaca Mulatta) trained to perform a rapid movement of flexion or extension of the contralateral forearm in response to an auditory cue. Bilateral degeneration of the SN was obtained by the systemic administration of MPTP. MPTP (0.35 mg/kg, i.v.) was injected daily for 4 consecutive days. Three series of injection were administered at 2 weeks intervals. Unit recordings were performed after complete recovery of the acute effect of the drug. **Results**. In normal animals, the majority of neurons in area 4 modified their discharge frequency before the onset of arm displacement (80 to 160 msec before) and returned to baseline level just after this onset (0 to 200 msec after). In MPTP-treated monkeys, the main observation was that numerous motor cortex neurons still modified their discharge before the onset of movement but did not stop with it and lasted well beyond the arm displacement (200 to 400 msec after). The behavioral reaction time is increased and the maximal velocity of the movement is drastically decreased. **Conclusion** These preliminary data are consistent with previous results reported in area 4 after a stereotaxic unilateral lesion of the SN in monkeys (Gross et al., Exp. Brain Res., Suppl. 7, : 181, 1983). It may be suggested that the disturbed discharge of area 4 may result in the long lasting movements in MPTP-treated animals and stereotaxic lesioned animals and would correspond, at the clinical level, to bradykinesia, one of the most invalidant trait of Parkinson's disease.

This study was supported by CNRS ERA 493, the INSERM N° 15 and the EPR Aquitaine.

340.10 IMPAIRMENT OF FASTEST VOLUNTARY MOTOR ACTIVITY IN BASAL-GANGLIA DISEASES V.Hömberg*,H.Hefter* and H.J.Freund Dept. of Clinical Neurology,Univ. of Düsseldorf, W.Germany (SPON: European Neuroscience Association)

Whereas bradykinesia is the most prominent clinical feature in Parkinson's disease (PD) it is only rarely mentioned in another degenerative basal ganglia disorder,Huntington's disease (HD),which is rather characterised clinically by the release of irregular rapid involuntary movements.Little research has been done on the impairment of voluntary motor activity in HD and how it relates to the abnormalities in PD. We therefore analysed fastest voluntary index finger activity in patient groups with PD (subdivided into tremulous (PDTR,N=10) and akineto - rigid (PDAR,N=15) subgroups), HD (N=22) and their offspring at risk to develop the disease later in life (HDRI,N=30), compared to matched controls (N=45).
Fastest alternating flexion/extension movements were recorded by an accelerometer taped to the tip of the index finger and the fastest alternation frequency (ALT) was determined from power spectra. Reaction times (RT) and contraction times (CT) for fastest possible non-targeted isometric contractions were determined in a simple reaction time paradigm.

ALT,RT and CT were slowed to about half the normal values in all disease groups.Adopting a criterion of 2 standard deviations around the age-related regression lines over the controls revealed abnormalities of these parameters also in 20-30 % of the clinically normal HDRIs.
In both PD and HD the normal increase of the rate of rise of tension with increasing amplitude of isometric contraction ,which keeps contraction time invariant over a wide range of contraction amplitudes , was disturbed to a similar degree.

PD and HD, however ,showed considerable differences in the shape of force trajectories : Wherea in HD force and EMG build-up were smoothly prolonged, in both PDTR and PDAR the prolongation of contraction time was caused by superimposed action-tremor-like oscillations at a frequency of 8-10 Hz,which disrupted the force development.

It is concluded,that impairment of fastest voluntary motor activity is a common feature in basal-ganglia disorders.It may be detected already in very early "subclinical" stages. Pathophysiological mechanisms causing this common bradykinesia however are different in disorders affecting primarily the dopaminergic input compared to those affecting intrinsic basal ganglia neurons.

340.11 SUBSTANTIA NIGRA: A SITE OF ACTION OF MUSCLE RELAXANT DRUGS. L. Turski*, M. Schwarz*, T. Klockgether* and K.-H. Sontag* (SPON: P.L. Herrling). Max-Planck-Inst. Exp. Med., D-3400 Göttingen, F.R.G.

The reticular part of the substantia nigra (SNR) constitutes an output station of the basal ganglia which interconnects striatum with ventromedial thalamus, superior colliculus and reticular formation. γ-Aminobutyric acid (GABA) is considered to subserve the transmitter role of these pathways. The substantia nigra receives also an excitatory cortical input. Within subcortical structures of the brain, the substantia nigra has been identified as one principal region that is involved in the propagation and generalization of seizures, and regulation of the muscle tone. In rats, functional inhibition of the SNR by GABA agonists leads to suppression of the tonic component of generalized convulsions and motor expression of kindled seizures. An activation of neurons of the SNR with GABA antagonists leads to catalepsy and to increase in the muscle tone of the hind limbs. The present studies were undertaken to explore the role of the substantia nigra in the mechanism of action of well established muscle relaxants, baclofen (BAC) and midazolam (MID), and drugs acting via GABA or excitatory amino acid receptors, muscimol (MSC), γ-vinyl-GABA (GVG) and (-)2-amino-7-phosphonoheptanoic acid (AP7). In the following we will describe the changes in the pathologically increased muscle tone in genetically spastic Wistar rats (Han:WIST; spa/spa), which exhibit tonic activity in the electromyogram (EMG) of the gastrocnemius (GS) muscle.

The activity in the EMG was recorded from the GS muscle of genetically spastic rats of either sex, at the age of 12 weeks, 100-140 g in weight, with pairs of teflon-insulated wire electrodes. The electrical signals were amplified, band-pass filtered, full-wave rectified and fed into an integrator. The microinjections into the SNR were performed through chronically implanted guide cannulae. The drugs were delivered in a volume of 0.5 µl at a rate of 0.1 µl/min.

Intranigral injection of saline, 0.5 µl, bilaterally, did not alter the spontaneous tonic activity in the EMG recorded from the GS muscle of mutant rats. Microinjections of BAC (0.0001-0.001 µmol), MID (0.001-0.015 µmol), MSC (0.00025-0.001 µmol) and AP7 (0.001-0.01 µmol), into the SNR, bilaterally, reduced the tonic activity in the EMG in a dose- and time-dependent manner. Microinjection of GVG, 0.04 µmol, into the SNR, bilaterally, decreased the tonic activity in the EMG of GS-muscle in a time-dependent way. The effect of GVG reached its maximum 10-20 h after intranigral injection and abated during 48-72 h. The depressant action of MSC and AP7 on the tonic activity in the EMG was antagonized by co-administration of bicuculline and N-methyl-D-aspartic acid into the SNR, respectively.

These results identify the SNR as a site of action of muscle relaxant drugs and convincingly demonstrate that a profound myorelaxant effect may be achieved from the supraspinal motor center.
This research was supported by a grant from the DFG (So 136/2-1/3-1).

340.12 EFFECT OF UNILATERAL KAINIC ACID LESIONS OF THE ENTOPEDUNCULAR NUCLEUS AND SUBSTANTIA NIGRA RETICULATA IN POSTURE AND MOVEMENT IN THE CAT. A.G.Russell, C.G.Kukulka, K.Kultas-Ilinsky. Physical Therapy Research Labs, and the Department of Anatomy, University of Iowa College of Medicine, Iowa City, IA 52240.

The cortical-striatal-pallidal/nigral-thalamic-cortical loop has been implicated in movement initiation and in the modulation of ongoing activity. The output of the entopeduncular nucleus (EPN) and the substantia nigra reticularis (SNr) represents the sum of the sensory-motor integration in the neostriatum. The loss of this inhibitory influence on the thalamus should be reflected in movement changes.

The purpose of the study was to investigate postural and movement disturbances induced by unilateral chemical lesioning of the SNr and the EPN. Cats were trained to perform a self-initiated forelimb task which involves pushing a bar press while assuming the quadrupedal position on the force table. Postural and movement vertical reaction forces under each paw were measured prior to and following the unilateral chemical lesions. Electrical activity of selected postural and movement muscles were monitored by chronically implanted fine wire electrodes. It was possible to measure the force changes which occurred in preparation for and during the movement.

Onset of force increases, sequencing of initial force changes, and peak force developments in the support limbs prior to and during the movement appeared unchanged for pre- and post-lesioned animals. Further analysis of movement time, movement velocities and EMG parameters are ongoing and changes in these values may be revealed. Correlation of the results of histological verification of the sites and extent of lesions with behavioral parameters will be presented.

The preliminary results suggest minimal postural and movement disturbances following unilateral lesions of the feline EPN and SNr nuclei.

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340.13 DEVELOPMENT OF THE STRIATONIGRAL PATHWAY REVEALS A POSSIBLE MECHANISM OF STRIATAL COMPARTMENTALIZATION. G. Fishell* and D. van der Kooy (Spon: L. Grupp). Department of Anatomy, University of Toronto, Toronto, Canada M5S 1A8

Recently we have shown that the perinatal distribution of cell bodies giving rise to the striatonigral pathway is clustered. These clusters closely match the distribution of dopamine islands present perinatally. Dopamine islands are an early marker for the patch/matrix compartmentalization seen in the adult striatum. In order to assess the developmental importance of the early striatonigral cell clusters we investigated the time course over which this pathway is established. Using the retrograde fluorescent tracer true blue, nigral injections were made in various pre or postnatal rats (embryonic day 16 to postnatal day 7). Rats were sacrificed 16 to 24 hours after injection. After E16 injections the few striatal cells having nigral projections were restricted to the ventro-lateral striatum. By E17 most neurons that had been generated in the neostriatum projected to the nigra and thus labeled cells were diffusely distributed throughout the striatum; although dense labeling in the ventro-lateral part of the subcallosal streak is already present. At E19 well defined clusters of labeled cells (in a large adjacent matrix of non-nigral projecting striatal cells) were seen throughout the striatum. A clear subcallosal streak (of cells) was visualized. Clusters of labeled cells were best delineated at birth and were clearly visible until postnatal day 4. The clustered striatonigral pattern became diffuse as the majority of striatal cells acquired nigral projections over postnatal days 5 and 6. By postnatal day 7 striatonigral projection cells were diffusely distributed throughout the striatum.

Two important observations emerge. First, the perinatal changes in the distribution of the striatonigral projection cells (from diffuse to patchy to diffuse) parallels the modifications occurring in the striatal dopamine islands. This suggests that a tight developmental relationship exists between the striatonigral cells and the reciprocal dopaminergic pathway. Second, the embryonic appearance of clustering in the striatonigral projections coincides with or precedes the earliest patch/matrix marker known previously. Moreover, the diffuse retrograde labeling of most striatal cells at E17 suggests that the patch component of the neostriatum is born first. Thus neuronal birthdate is likely an important mechanism in organizing striatal compartmentalization.

340.14 ELECTROPHYSIOLOGICAL AND BEHAVIORAL ACTIONS OF DYNORPHIN IN THE SUBSTANTIA NIGRA J.M. Walker and M.W. Friederich, Department of Psychology, Brown University, Providence, Rhode Island 02912.

The cloning and sequence analysis of prodynorphin, established the third gene family of opioid peptides. These neurons apparently secrete several opioid products (α-Neo-endorphin, dynorphin, rimorphin) and possibly some non-opioid peptides as well (bridge peptide, C-peptide). The presence of dynorphin immunoreactivity in the substantia nigra (SN) pars reticulata, and its disappearance following ibotenic acid lesions to the caudate nucleus, strongly suggests the existence of a prodynorphin striato-nigral pathway.

Indeed, striato-nigral projections have been investigated for over forty years, and are widely believed to secrete substance P (and related products from the substance P precursor) as well as GABA. It has been hypothesized that striato-nigral projections provide feedback to the SN pars compacta. It has also been suggested that striato-nigral fibers serve as output cells from the basal ganglia, forming connections in the SN pars reticulata in route to lower motor structures.

The roles of the products of prodynorphin in the SN pars reticulata have been under investigation in our laboratory. We found that dynorphin and any number of opiate and non-opiate fragments of dynorphin induce contralateral rotation in rats after microinjection in the SN pars reticulata. This effect is markedly reduced by haloperiodol, and the effects of 6-hydroxydopamine lesions are under investigation.

Electrophysiological studies have also been carried out using extracellular recording with administration of prodynorphin products locally using micropressure-ejection methodology. Dynorphin-17 and Dynorphin-8 fail to have direct effects on substantia nigra pars compacta dopamine (DA) cells. ZR-DA interneurons described by Grace and Bunney were identified just ventral to the pars compacta by an increase in firing rate in response to foot pinch. These cells respond to dynorphin with a decrease in firing rate. The effects of dynorphin on these cells resemble the effects of several other opiates in this area. In the deeper zona reticulata mainly excitatory effects are seen. The nature of these excitatory actions are under investigation.

The data suggest that dynorphin disinhibits DA cells by way of an interneuron in the zona reticulata. An apparently non-opiate action in the deeper areas of the zona reticulata is consistent with opiate insensitive actions on rotational behavior, but the physiological relevance of this action remains to be determined.

340.15 ASYMMETRIC DOPAMINE RELEASE IN THE SUBSTANTIA NIGRA OF RATS RUNNING ON A CURVED TURNTABLE TREADMILL. B.K. Yamamoto and C.R. Freed Univ. of Colo. Med. Ctr., Denver, CO 80262.

We have shown that rats trained to circle have increased dopamine (DA) turnover in substantia nigra ipsilateral to the circling direction. In a reciprocal manner, DA release and turnover in caudate was increased in the contralateral side. Furthermore, rats forced to run on a curved turntable treadmill show a contralateral increase in caudate DA that is proportional to both running speed and body arc. We now have studied DA release in the substantia nigra pars compacta (SNc) of rats forced to run in place on a curved turntable treadmill. Male Sprague Dawley rats weighing 250-300 g were bilaterally implanted with 200 μm in vivo electrochemical carbon paste electrodes into the SNc. One day following implantation, signals were measured by linear sweep voltammetry at a rate of 10mV/sec every 5 min using a DCV 5 voltammetry amplifier with semi-derivative signal processing. Following a one hour baseline stabilization period, rats were placed on the treadmill and forced to run for one hour.

Contralateral and ipsilateral refer to the side relative to the postured running direction. Electrochemical signals are represented as percent of baseline. N=6

| | BASELINE | RUNNING (minutes) | | POST-RUNNING BASELINE |
|---|---|---|---|---|
| | 0 | 30 | 60 | 180 |
| Contralateral | 100 ± 1 | 85 ± 5* | 90 ± 2 | 96 ± 3 |
| Ipsilateral | 100 ± 1 | 104 ± 3 | 111 ± 3* | 101 ± 2 |

*p<0.05

In separate groups of animals, changes in the signal during running were abolished by pre-treatment with 300 mg/kg αmethyl-p-tyrosine (αMPT). Further experiments showed that the MAO inhibitor, pargyline (75 mg/kg) decreased the signal by 15%. Subsequent administration of αMPT decreased the signal by an additional 25%. The combination thus produced a total decrease of 40% (p<.01). αMPT alone produced a comparable decrease of 35% (p<.01). Therefore, the catecholamine signal in the SNc represents both DA and DOPAC.

In conclusion, these results are in agreement with electrophysiological studies showing that DA is inhibitory on SNc cell firing resulting in decreases in caudate DA release. Therefore, during curved treadmill running, an increase in DA release in ipsilateral SNc may represent inhibition of nigral cell firing with a reciprocal decrease in release of caudate DA. Conversely, a decrease in release in contralateral SNc increases nigral firing and increases DA release in the caudate on that same side.

340.16 TRANSPLANTATION OF CULTURED FETAL HUMAN ADRENAL CHROMAFFIN AND SPINAL CORD CELLS TO RAT BRAIN, H. Kamo[1]*, S. U. Kim[2]*, P. L. McGeer[1] and D.H. Shin[2]* (SPON. John G. Sinclair). [1]Kinsmen Laboratory of Neurological Research and [2]Division of Neurology, Depts. of Psychiatry and Medicine, University of British Columbia Vancouver, B.C. V6T 1W5, Canada.

The loss of specific neuronal groups has been reported in Parkinson's disease, Huntington's disease and Alzheimer's disease. Drug therapy either does not exist or only provides symptomatic relief. One approach to compensate for these neuronal losses might be the transplantation of a critical mass of neurons, capable of producing appropriate neurotransmitters, into the involved anatomical sites. For this to be feasible in human disease, it would appear best to use cultured human cell lines. This abstract reports preliminary studies in which such cultured human cell lines were transplanted into lesioned adult rat brains.

Adrenal chromaffin cells, obtained from a therapeutically aborted human fetus of about 11 weeks gestation, were cultured for three weeks in vitro with exposure to NGF to transform them into catecholaminergic neurons. They were transplanted to the left striatum of rats which had been subjected 1-2 weeks previously to a unilateral 6-hydroxydopamine lesion of the left nigro-striatal tract. Transplanted cells became established through strands of tissue growing into the host striatum. No signs of inflammation or rejection were observed up to the time of sacrifice one month post-tranplantation. Histofluorescence examination of the implanted areas showed many clusters of cells having an intensely positive catecholamine fluorescence with some of the cells developing conspicuous processes.

In similar studies the septal-hippocampal pathways in rats was surgically lesioned in the hippocampal fimbria. Cultured spinal cord neurons from a therapeutically aborted human fetus were subsequently implanted just posterior to the lesion. Possible cholinergic reinnervation of the hippocampus is being studied by acetylcholinesterase histochemistry, choline acetyltransferase immunohistochemistry and biochemical assays for choline acetyltransferase. This study employs the techniques of Bjorklung and Sveni (Cell Tissue Res. 185: 289-302, 1977) but is the first to report transplantation of cultured human cell lines.

This study, showing survival of such cultured human cells transplanted into rat brain tissue, might indicate the feasibility of using cultured human material for future human neuronal transplantation studies as a therapeutic measure.
(Supported by grants from the British Columbia Health Care Foundation, the MRC of Canada, the Mr. and Mrs. P.A. Woodward's Foundation and the B.C. Medical Services Foundation).

340.17 SINGLE UNIT ANALYSIS OF THE VENTRAL TIER OF LATERAL THALAMIC NUCLEI IN PATIENTS WITH PARKINSONIAN TREMOR. F.A. Lenz*, R.R. Tasker*, H.C. Kwan, S. Schnider*, R. Kwong* and J.T. Murphy. Departments of Neurosurgery, Physiology and Electrical Engineering, University of Toronto, Toronto, Canada.

Cells which fire in bursts of frequency similar to that of tremor ('tremor cells') have been described at the thalamic lesion site for relief of parkinsonian tremor. In this study, the functional properties of these cells were defined by single unit analysis at the time of stereotactic surgery in an attempt to understand the mechanism of parkinsonian tremor.

In the ventral tier of lateral thalamic nuclei, sensory cells were found which reproducibly responded to specific modalities of sensory stimulation in well defined receptive fields. Anterior to these cells, at the optimal lesion site in parkinsonian tremor, three cell types were encountered: 1) voluntary cells which significantly increased their activity in advance of specific voluntary movements, 2) combined cells with voluntary and sensory properties linked so that sensory stimulation produced movement in the direction opposite to the voluntary movement related to cellular activity, and 3) no response cells with neither voluntary nor sensory activity.

Many of these cells had a large amount of activity at tremor frequency and were significantly correlated with tremor as tested by spectral cross-correlation analysis, with evaluation of the coherence function. Therefore, on the basis of location at the lesion site and correlation with tremor, these cells were identified as potentially driving tremor. During tremor, the latency between thalamic and EMG activity was measured from spike triggered averages (STA) of EMG activity. Conduction delays for transmission from thalamus to muscle via motor cortex were estimated to be 20-30 ms or, if experimental errors in the STA technique are included, 0-50 ms. Only in the case of combined cells did a majority of the cells have latencies in the 0-50 ms range. These results suggest that combined cells drive tremor by involvement in an unstable long loop reflex arc traversing movement receptors, thalamus, motor cortex and spinal motor neurons.

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340.18 ARE POSTURAL AND AGONIST MUSCLES AFFECTED SIMILARLY IN PARKINSON'S DISEASE? M.W. Rogers* (Spon: D.W. Baxter). Physical Therapy Res. Labs., College of Medicine, Univ. of Iowa, Iowa City, IA 52242, and School of Physical and Occupational Therapy, McGill University, Montreal, Quebec, Canada H3G 1Y5

Single unit recordings have indicated that activity of the basal ganglia precedes and accompanies both ballistic and slow movements of the limb. In addition, the earliest task related EMG changes have been observed in the postural (paraspinal) musculature in advance of the limb muscles over a range of movement velocities (DeLong and Strick, Brain Res. 71: 327, 1974). Given the abnormalities in movement speed and postural stabilization manifested by Parkinson's patients, this study investigated whether the control of postural (biceps femoris=BF, erector spinae=ES) and agonist (anterior deltoid=AD) muscles might be affected similarly in patients with Parkinson's disease in association with arm movements performed at different speeds.

Nine idiopathic Parkinsonians and age-matched controls performed standing unilateral shoulder flexion movements in response to a light flash which followed a warning signal, at each of 3 different speeds (S=40±8 deg./s., I=80±16 deg./s., F='as fast as possible'). Movements made at F velocities were executed under a simple reaction time (SRT) paradigm. Movement onset (MO), movement time (MT), EMGs, and segmental body sway were recorded simultaneously.

Analysis revealed that for both subject groups: (1) The postural muscles were usually activated before the agonist during F speed movements, but were recruited fewer times and later with respect to AD as MT became slower; (2) Pre-movement EMG duration decreased for ES but not BF as a function of increasing MT; (3) The mean EMG onset times for postural muscles relative to agonist activation and MO were comparable between groups.

In contrast to controls, EMG patterns of the postural muscles in Parkinsonians were characterized by intermittent activity, and shorter pre-movement durations for ES ($p<.01$) and BF ($p<.05$) across all speeds. A similar discontinuity of EMG activity was observed for the agonist during shoulder flexion at S and I velocities, while that of F speed was often accompanied by segmented bursts of normal duration.

Under the simple reaction time condition, akinetic Parkinsonians (n=4) with prolonged SRT ($p<.05$) demonstrated delays in EMG onset latencies for AD ($p<.05$), ES ($P<.05$), and BF ($p<.10$).

These results suggested that the normal control of both postural and agonist muscles may be altered similarly in Parkinson's disease. In particular, parallel changes in postural and agonist muscle activation patterns may indicate a disruption of a common drive to functionally linked muscle synergists over a range of movement speeds.

Supported by a grant from the Foundation for Physical Therapy.

## DISORDERS OF MOTOR SYSTEMS AND NEURAL PROSTHESES

341.1 MICTURITION INDUCED IN PARAPLEGIC CATS BY REFLEX INHIBITION OF THE EXTERNAL URETHRAL SPHINCTER. P.W. Ruenzel,* F.A. Jolesz,* and E. Henneman. (SPON: M. Moore-Ede). Dept. of Physiology and Biophysics (Harvard Medical School) and Dept. of Radiology (Brigham & Women's Hosp.) Boston, Mass. 02115.

It was previously reported (Jolesz et al., Science 216: 1243, 1982) that in paraplegic cats by recording motor nerve electrical activity in the pudendal nerve branch which supplies the external urethral sphincter (EUS) all the reflex responses characteristic of a flexor reflex could be demonstrated. We also found that in paraplegic rats single motor unit EMG activity in the external anal sphincter was similar and roughly parallel to that in the hindlimb flexor muscles. This suggested that limb muscle reflexes might be evoked to inhibit EUS overactivity (which occurs in paraplegia and many spinal injuries) if the pudendal nerve on one side had previously been severed, leaving the EUS under unilateral reflex control. We now report that we have induced micturition in paraplegic cats by performing limb maneuvers that evoke reflexes inhibitory to the motoneurons supplying the EUS on the innervated side.

Under ether anesthesia the spinal cords of cats of either sex were transected at the mid-thoracic level by extra-dural ligation. A 3-5 mm segment of the right pudendal nerve was extirpated, leaving the EUS innervated only on the left. 50 ml of Renografin-60 radio-opaque contrast material was infused I.V. On the following day the animals were continent, and they had not voided. Fluoroscopic examination revealed an overfull bladder and a closed EUS. Manual pressure on the bladder did not overcome EUS resistance to urine flow.

The following maneuvers, all inhibitory to motoneurons supplying flexor muscles in the left hindlimb, were performed: (1) left hindlimb was placed in full flexion, stretching the extensors and reflexly causing inhibition of the motoneurons supplying the left side of the EUS, (2) right hindlimb was placed in full extension, stretching the flexors and causing crossed inhibition of the motoneurons supplying the left side of the EUS, (3) footpad of right hindlimb was pinched, evoking a crossed extension reflex of the left hindlimb and crossed inhibition of the motoneurons supplying the left side of the EUS. On fluoroscopic examination these maneuvers caused relaxation of the bladder neck, and when the bladder was squeezed, urine was seen to pass freely down the urethra. Although this approach solves the problems caused by spasm of the external sphincter, active contraction of the bladder (induced at the same time) would be of further advantage in obtaining complete emptying of the bladder.

Supported by the Spinal Cord Research Foundation of the Paralyzed Veterans of America.

341.2 INFORMATION TRANSFER RATES IN ELECTROCUTANEOUS SPATIAL POSITION FEEDBACK CODES. R.R. RISO AND A.R. IGNAGNI*. Rehabilitation Eng. Program, Case Western Reserve Univ., Cleveland, OH, 44109

Electrocutaneous communication techniques are being developed to provide substitute and augmentative sensory feedback for users of Functional Neuromuscular Stimulation (FNS) systems that restore movement to otherwise paralyzed and asensory extremities. As the basis for this feedback technique, coded electrical stimuli are applied to the user's skin in an area where sensation is normal. One specific application is to give feedback information about shoulder protraction-retraction movements which spinal injury patients currently use to control FNS orthoses that provide functional grasp. A linear array of electrodes is used to form a display in which the relative position of the shoulder is signaled by activating a corresponding electrode site within the array. A sternum mounted joy stick controller that is connected to the shoulder via a telescoping wand is used to track the user's shoulder movements. An important issue with regard to the design of the electrocutaneous display is the number of electrode elements to use: Theoretically, the amount of information transferred by a code increases as the number of possible code words is increased. Thus, a spatial position code which utilizes eight electrodes would transfer more information per stimulus presentation than, for example, four electrodes. The number of electrode sites dictates the absolute resolution that could be provided about the position of the shoulder, however, subjects require increased decision time in order to "track" or interpret codes as the number of possible code words increases. This restriction serves to diminish the information transfer rate (ITR). We computed the rates of information transfer from specially designed psychomotor tasks. Experiments were performed on 4 normal adults and results compared for electrode displays consisting of 4,5,6,7 and 8 stimulation sites. Electrodes 1-4 were located axially on the upper arm and 5-8 were placed horizontally on the upper back. Electrodes were spaced 40 mm apart on both the arm and back skin, and S's were pretrained to correctly identify any randomly activated electrode with 90% surety. S's identified each randomly activated electrode by releasing a reaction time response key and then depressing one of N numbered keys to register their choice. S's were told in advance of each portion of the experiment whether the display set would consist of the elements 1-4,1-5,1-6 or 1-8. The mean reaction times (MRT) for recognition of N electrode sites averaged across all subjects are given below. Also presented are the associated mean computed information transfer rates. Data are corrected for increases in reaction time engendered by S's having to choose among the N response keys. Supported by NIHR Grant #G008300118.

| | N=4 | N=5 | N=6 | N=7 | N=8 |
|---|---|---|---|---|---|
| MRT (msec + SE) | 626 + 4 | 623 + 4 | 751 + 5 | 902 + 8 | 930 + 8 |
| ITR (bits/sec + SE) | 3.01+.01 | 3.57+.01 | 3.30+.01 | 3.01+.01 | 3.12+.01 |

341.3 REACTION TIME AND MOVEMENT TIME DEFICITS IN PARKINSONIAN PATIENTS. C.J. Hunker* and J.H. Abbs* (SPON: R. Netsell). Boys Town National Institute, Omaha, NE 68131 and Speech Motor Control Labs, University of Wisconsin, Madison, WI 53705.

Even though a number of investigators have shown that the temporal aspects of voluntary limb movements in patients with Parkinson's disease were impaired, there appears to be considerable individual variation in these performance deficits. Some parkinsonian subjects have been found to be quite slow in movement initiation (akinesia) and execution (bradykinesia) whereas others have shown normal response initiation and/or movement times. In an effort to further understand these phenomena, the initiation and duration of orofacial and index finger movements were examined relative to characteristic symptoms. Reaction time movements and agonist EMG from the lips, tongue, jaw and index finger were recorded from two groups of parkinsonian subjects (with and without resting tremor), as well as from a normal control group.

Subjects identified clinically as predominately tremorous exhibited more impaired reaction times (RT) than their rigid counterparts. In examining movement times (MT) of the specific subject groups, it was found that the rigid subjects were more impaired in movement durations than the tremorous group. When RT and MT were examined as a function of the clinically defined groups, they were inversely related. In the tremorous group, prolonged RTs were present in conjunction with near normal MTs while in the rigid group normal RTs were present in conjunction with prolonged MTs.

These data support the hypothesis of Evarts et al. (Brain, 104: 167, 1981) that impairments in voluntary movement initiation and execution may be mutually exclusive. The results presented here suggest that the pathological mechanisms involved in tremor and muscle rigidity may account, in part, for the movement aberrations of akinesia and bradykinesia, respectively. Research supported by a grant from NIH (NS 22318).

341.4 CONTROL OF SPEECH TIMING FOLLOWING BASAL GANGLIA AND WHITE MATTER LESIONS. C.L. Ludlow, N.P. Connor*, and J. Rosenberg*. Human Motor Control Section, MNB, NINCDS, Bethesda, MD 20205.

Neurological diseases involving the basal ganglia have been found to impair control of syllable execution time (Huntington's Disease) and sentence execution time (Parkinson's Disease) (Ludlow, Connor & Bassich, Neurosci. Abstr.10:906, 1984). Our purpose was to study speech timing when lesions involve both the basal ganglia and white matter tracts. Patients with penetrating missile wounds involving both the basal ganglia and white matter tracts on the right or left side were studied 15 years post-lesion. In conversation, all patients presented with a speech rhythm and rate disorder; speech dysprosody (SD). Experimental tasks were administered to determine: 1) whether simple reaction time, syllable repetition rate and control of execution time were impaired in the SD patients; 2) whether speech timing task performance differed between SD patients and patients without white matter tract involvement; and, 3) whether the normal inter-relationships between different aspects of speech timing were found in SD patients. Measures made from sound spectrograms of speech during experimental tasks included: reaction time following a click; syllable repetition rate; syllable, word, phrase, pause, and sentence execution time; and changes in syllable, word, pause, phrase and sentence execution times with alterations in speech rate and syllable stress. The SD patients were significantly impaired ($p \leq .05$) on: reaction time, syllable repetition rate and control of syllable initiation time during stress contrasts. This group was not impaired on any of the execution time measures or measures of change in execution time. The normal relationships between syllable repetition rate and change in syllable initiation time for stress were not found in the SD patients. Change in syllable initiation time for stress was related to sentence execution time and execution time change only in the SD patients. In basal ganglia disease, these deficits were not found, and reaction time did not relate to other aspects of speech timing. In conclusion, difficulties with syllable initiation were characteristic of SD patients with basal ganglia and white matter damage, but were not found in patients without white matter involvement.

341.5 THE GENERATION OF A RHYTHMICAL 3-4 HZ CEREBELLAR TREMOR REQUIRES MUSCLE STRETCH. J. Hore and D. Flament. Dept. of Physiology, Univ. of Western Ontario, London, Ont., Canada N6A 5C1.

One classic sign of a lesion of the lateral cerebellar nuclei is a rhythmical oscillation of the limb at about 3-4 Hz (cerebellar intention tremor). This tremor can occur when moving a limb or when attempting to hold it stationary. Two theories exist which could explain this tremor. The first is that tremor results from a central neural network behaving as an oscillator (eg. tremor still occurs after dorsal rhizotomy[2,3]). The second is that tremor results from unstable long loop stretch reflexes (eg. tremor is strongly influenced by proprioceptive feedback[1]).

To provide further information about cerebellar tremor 3 Cebus monkeys were trained to perform a step-tracking task under isotonic and isometric conditions. In the isotonic situation monkeys moved a handle against different torques between 2 targets displayed on an oscilloscope and separated by about 40°. In the isometric situation the handle was clamped and the 2 targets represented different levels of force. These tasks were performed during normal conditions and during cerebellar dysfunction produced by reversibly cooling through 2 probes implanted on either side of the dentate nucleus.

Under normal conditions monkeys performed both isotonic and isometric tasks smoothly and without tremor. In the isotonic situation during cerebellar cooling a marked 3-4 Hz rhythmical oscillation occurred when attempting to hold the arm stationary following movements in all 3 monkeys. In the isometric task during cerebellar cooling records of force became irregular as the monkeys were unable to hold force steady at the new level. Oscillations occurred in some trials when attempting to hold force steady, but they were more irregular and at a lower frequency (2-3 Hz) than the oscillations in the isotonic situation. Furthermore, tremor following isotonic movements was synchronized to end of movement as previously reported[1] while oscillations following isometric contractions were not synchronized to the end of the contraction.

The results indicate that peripheral feedback resulting from muscle stretch is necessary for the generation of a rhythmical 3-4 Hz cerebellar tremor.

[1]Flament D, Vilis T, Hore J. Exp. Neurol. 84: 314, 1984.
[2]Gilman S, Carr D, Hollenberg J. Brain 99: 311, 1976.
[3]Liu CN, Chambers WW. Acta Neurobiol. Exp. 31: 263, 1971.

This work was supported by the MRC of Canada, MT-6773.

341.6 THE INFLUENCE OF CONDITIONING STIMULATION OF THE COMMON AND SUPERFICIAL PERONEAL NERVES ON THE SOLEUS H-REFLEX IN NORMALS AND IN SUBJECTS WITH SPASTICITY. M. Levin*, A.B. Arsenault* and C.E. Chapman. Ecole de réadaptation, Univ. de Montréal and Institut de réadaptation de Montréal, Montréal, Québec, Canada, H3C 3J7.

In patients with spinal spasticity the soleus Hoffmann (H) reflex recovery curve conditioned by tibial nerve stimulation shows characteristic changes from the normal situation. Changes have also been reported in the soleus H-reflex conditioned by common peroneal nerve (CPN) stimulation although only delays of up to 30ms have been investigated in detail. This study has looked at longer delays (10-2000ms), comparing soleus H-reflex recovery curves conditioned by stimulation of the CPN and its cutaneous branch, the superficial peroneal (SPN), in 10 normal subjects and in 11 subjects with spasticity due to a clinically complete traumatic lesion of the spinal cord. The results were correlated with a clinical evaluation of spasticity. The conditioning stimulus was a train of 3 pulses (pulse duration 0.2ms) at 100Hz, repeated every 10s. The intensity was 1.4 X motor threshold (CPN) and 1.5 X threshold for a compound sensory action potential (SPN). The test stimulus (1ms pulse) was adjusted to produce an H-reflex, 75% of maximum.

In normal subjects, H-reflex recovery curves conditioned by the CPN showed (1) a large decrease at delays of 10-100ms, (2) a rapid recovery peaking at 140-160ms, (3) a slight decrease at 400ms and (4) a gradual recovery towards control levels which was still incomplete at 2s. Stimulation of the SPN had only facilitatory effects, two peaks occurring at about 40 and 140ms. In spastic subjects, the effects of CPN stimulation varied. In 6 subjects with moderate/severe spasticity, there was an early decrease and rapid recovery as in normals but the recovery was usually faster and earlier. There was little evidence of the late peak (at 140ms) and subsequent depression as in normals. In 4 subjects with mild spasticity, the H-reflex showed a gradual and modest decline (onset: 20ms, end: 600ms), followed by a slow recovery to control values. SPN stimulation caused, if anything, a slight decrease of the soleus H-reflex in the spastic subjects.

The results suggest that muscular, but not cutaneous, afferents are responsible for the inhibitory effects of CPN stimulation on the soleus H-reflex. Cutaneous afferents may contribute to the late peak at 140ms, perhaps through a supraspinal loop since these facilitatory effects were absent in the spinal subjects. The results also suggest that the shape of the soleus H-reflex recovery curve conditioned by CPN stimulation may give an indication of the severity of the spasticity. Supported by the FRSQ and FCAC.

341.7 SPASTIC LOCOMOTION:A DYNAMIC ANALYSIS OF STROKE PATIENTS BEFORE AND AFTER THERAPY. C.J. Winstein* and A. Garfinkel* (SPON: J.L.Smith). Dept. of Kinesiology, University of California, Los Angeles, CA 90024.

Following therapy, stroke patients demonstrated improvements in specific gait measures (Winstein, C.J. and Perry, J., Phys Ther., submitted, 1985). It was suggested that a possible source of this improvement was a reduction of interfering spasticity. Typically, spasticity is evaluated clinically with passive limb movement. This method is clearly not appropriate for assessing spasticity in motion. Qualitative dynamics offers a possible approach to describing spastic limb motion (Garfinkel, A., Am. J. Physiol. 245: R455-R466, 1983). The following knee and ankle motion analyses examined spastic locomotion and the changes which occurred following therapy. Qualitative dynamics and associated quantitative indices were compared.

Stroke patients walked on a 15 meter walkway at a self-chosen comfortable velocity before and after a three week therapy program**. Signals from two electrogoniometers (double-parallelogram design) and two footswitches were recorded. The footswitch signals were used to define the gait cycle variables (gait cycle time, stride length, single limb stance time, etc.). Electrogoniometric signals were digitized (625 samples /sec). These digitized data were sampled every 20 msec, smoothed and then processed by a PDP 11/34 computer. Intersegmental knee-ankle coordination plots and phase plane plots of joint position as a function of an estimate of the first order derivative were generated.

Preliminary results indicate that while several subjects showed large improvements in linear velocity, they demonstrated quite divergent changes in qualitative dynamics. In particular, of two subjects who showed similar quantitative changes, one developed a totally different intersegmental coordination pattern, while the other displayed an intersegmental coordination pattern that was similar to the pre-treatment pattern, but scaled up in size. The phase plane plots revealed a dominant velocity oscillation which had a relatively smaller amplitude post-treatment. This finding suggests that dynamic spasticity was not itself reduced, but that its relative interference to joint motion was reduced due to a concomitant increase in angular joint velocity.

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341.8 INHIBITION OF PROSTAGLANDIN SYNTHETASE PRODUCES A MUSCULAR DYSTROPHY-LIKE MYOPATHY. I.S. McLennan* (SPON: R. Mark). Dept. of Behavioural Biology, Research School of Biological Sciences, Australian National University, Canberra, ACT, 2601, Australia

The function of striated muscle is dependent upon the highly organised nature of its myofibrils. Disruption of myofibrillar structure is part of the aetiology of many muscle pathologies, including various forms of muscular dystrophy. The regulation of myofibrillar assembly is therefore of interest. I report here that administration of inhibitors of prostaglandin synthetase to chicken embryos disrupts the development of their myofibrils and creates a pathology similar to that of muscular dysgenesis and muscular dystrophy.

Chicken embryos were treated with either aspirin (2.7mg) or indomethacin (0.17mg) when 4 days old and sacrificed when either 10 or 19 days old. Their hindlimbs were skinned, fixed in a solution containing 2.5% glutaraldehyde, 4% formaldehyde and 0.1M sodium cadodylate, pH 7.4. Their Iliofibularis were then dissected from their thighs, postfixed with osmium tetroxide, stained en block with uranyl acetate and embedded in araldite. Light microscope sections were stained with toluidine blue and electron microscope sections with lead citrate.

The myofibrillar structure of the treated muscles was grossly and variably distorted. In the extreme, the myofibrils had totally dissociated. More frequently, the myofibrils had formed but were malaligned and had a variety of distorted structures, including disruption of the Z band, lack of electron density in the H zone, depletion of thick or thick and thin filaments and inappropriate splitting.

These results indicate that prostaglandin E1 is an important regulator of myofibrillogenesis and that abnormal prostaglandin function may directly or indirectly be involved in the pathogenesis of diseases such as muscular dystrophy and muscular dysgenesis. Prostaglandin E1 may therefore have therapeutic value in ameliorating the loss of function associated with many muscle pathologies.

341.9 VALIDATION OF AN ANIMAL MODEL OF IMPAIRED MUSCLE GLYCOLYSIS: IODOACETATE DOES NOT DIRECTLY ALTER EXCITATION-CONTRACTION COUPLING. R.L.RUFF. Dept. of Neurology, Cleveland VAMC and Case Western Reserve University, Cleveland, Ohio 44106.

There are several inherited disorders of muscle glycolysis and glycogenolysis. In these diseases ischemic exercise results in severe muscle cramping and sometimes rhabdomyolysis. The muscle cramping is electically silent, suggesting that ischemic contraction produces either excessive release of calcium from the sarcoplasmic reticulum (SR) or blocks the ability of the SR to uptake calcium. An understanding of the molecular basis of the contracture could suggest specific treatments and might also provide additional insights into the common problem of muscle cramping. Because these diseases are rare and human tissue is difficult to obtain, an animal model would be useful. Brumback et al. (Muscle and Nerve 6:52,1983) showed that intra-aortic injection of iodoacetate, which blocks the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase, produced exercise-induced, electrically silent muscle contraction and rhabdomyolysis. There were no systemic complications from the injections so that muscle changes could be studied in vivo. However, for this model to be valid, iodoacetate must not directly affect excitation-contraction coupling.

The actions of iodoacetate were studied in intact and chemically skinned muscle fibers from the extensor digitorum longus (EDL) muscle in adult (300g) Sprague Dawley rats. Membrane potential, input resistance, and electrical excitability of muscle fibers studied in vivo were not altered by arterial injection of iodoacetate followed by ischemic stimulation resulting in prolonged muscle contracture. The strength-duration relationship of individual fibers studied in vitro were not changed by treatment with iodoacetate. Iodoacetate did not cause spontaneous contraction, change the pCa-tension relationship, or alter the normalized tension of skinned muscle fibers. It did not cause calcium release from the SR, or prevent calcium accumulation by the SR. Therefore, iodoacetate did not appear to have any direct effects on the sarcolemma, the myofibrillar proteins, or the SR. Thus it appears that iodoacetate produces exercise-induced contracture by blocking muscle glycolysis which results in depletion of high energy stores

341.PO DYSFUNCTION OF VOLUNTARY MOVEMENT IN SCHIZOPHRENIA. P.B. Vrtunski, D.M. Simpson*, K.M. Weiss* and G.C. Davis*. Cleveland VA Medical Center (Research Service), Brecksville, OH 44141.

A number of disturbances of motor function have been observed in schizophrenia. The most extensive work was done on the eye tracking dysfunction (ETD) by Holzman and his collaborators. Voluntary movement involving skeletal musculature was not systematically investigated, however. The present study involves the use of reaction time paradigm to generate a button-press response which is then subjected to microbehavioral analysis. The analysis is carried out by recording the force analogs which make up the actual response, dividing it into premotor and motor segments, and observing whether the tasks of varied difficulty differentially modify movement performance.

The control group (N=20) with mean age of 30.6 years, and the schizophrenic group (N=19) who met DSM-III criteria with mean age of 33.0 years, were compared. Four types of stimuli were used, varying in modality of presentation (visual or auditory), and type of material (verbal or nonverbal). Thus, the task involved a standard choice reaction time procedure in which the subject made a forced choice discrimination with lines, pictures, sounds and spoken words (Vrtunski, P.B. et al. Brain, 106:929, 1983).

Schizophrenics had significantly longer premotor segments, 1020 msec overall, than the control subjects, 659 msec overall ($F=22.03$, $p<.0001$). The four stimulus conditions differentially affected this variable ($F=138.96$, $p<.0001$), with line discriminations being accompanied by the shortest premotor segments and word disciminations, by the longest. The group by stimulus condition interaction term was not significant. Schizophrenics exhibited significantly longer motor segments (353 msec overall) than controls (207 msec overall) ($F=16.38$, $p<.001$). Duration of motor segment also increased significantly with task difficulty ($F=11.64$, $p<.0001$). Most importantly, there was a significant group by stimulus condition interaction ($F=3.58$, $p<.02$) with schizophrenics showing longer motor segments after verbal discriminations with either visual or auditory stimuli.

Although increased duration of movement performance in the isometric button-press may, like disordered visual tracking, not prove specific for schizophrenia (Holzman, P.S. Schizoph. Bull., 9:33, 1983), our results provide a method for examining questions of the cognitive deficits in schizophrenia and their interaction with psychomotor organization. The large deterioration produced in motor response with verbal discrimination tasks in schizophrenics suggests the impairment of specifically verbal information processing.

342.1 EFFECT OF FAR ULTRAVIOLET RADIATION (EXCIMER LASER) ON THE ADULT SPINAL CORD OF RAT. H. O. Nornes, R. Srinivasan*+, R. Solanki*, and E. Johnson*. Departments of Anatomy and Electrical Engineering, Colorado State University, Fort Collins, CO 80523, +IBM Watson Research Center, Yorktown Heights, N.Y. 10958

Transplantation methods have possibilites for reconstruction of the injured central nervous system (CNS), and we are investigating the possibility of using an excimer laser as an ideal surgical tool which causes minimal damage to the surrounding CNS tissue and does not coagulate and seal the surgical surface. This laser emits ultraviolet radiation at 193 nm which interacts photochemically with organic polymers and breaks the molecules into smaller volatile fragments (photoablation) without heating the surrounding material (B. G. Garrison, R. Srinivasan, J. of Applied Physics, 55: 1985).

A laminectomy was performed on 8-10 week old Sprague/Dawley female rats at $T_{10}$, and the spinal cord was exposed to 193 nm radiation generated from argon-flouride laser. A cavity was made in the middorsal region about 1-3 mm deep and 1-2 mm in diameter. Five weeks post-surgery, the animals were analyzed with the light microscope (Bodian silver and hematoxylin, eosin, phyloxine stains.

The cavities in the spinal cord became filled with loose fibrous connective tissue, while the edges had sharp boundaries along both white and grey matter. In some cases there was no evidence of necrotic tissue or hyperplasia of glia cells in either the white and grey matter. The cuts with little or no visible tissue damage had extensive regeneration of nerve fibers across the boundary and into the loose fibrous scar. This regeneration occurred in both the grey and white matter and was in sharp contrast to the response in cavities made with knife-cuts or suction lesions. Those edges were irregular and exhibited necrosis and hyperplasia, and there was little or no regeneration of host fibers across the host-scar boundary.

The unique finding of this study was the extensive regeneration of spinal cord axons across the host-scar boundary into the loose fibrous scar in the tissue cut with the laser. These results raise the possibility that the excimer laser may be an ideal tool for reconstructive surgery in the central nervous system.

(Supported by Spinal Cord Society and IBM Watson Research Center)

342.2 THE MODIFIED VASCULAR CLAMP TECHNIQUE: A NEW EXPERIMENTAL MODEL FOR SPINAL CORD INJURY. E. C. Benzel and M. M. Thomas. Louisiana State University School of Medicine, Shreveport, LA, 71130.

The evaluation of therapeutic modalities for the treatment of spinal cord injury (SCI) has been heavily influenced by animal models involving the application of a dorsally applied injury. Although these models are both easily performed and quantitable they do not offer an accurate reproduction of the mechanisms of injury in most SCI clinical situations. Most patients who incur a traumatic SCI do so by way of a ventrally located force (mass) impinging upon the spinal cord. This force (mass) persists following the initial impact. The standard weight-drop technique of SCI fails in mimicking the clinico-pathological situation in several ways: First the force is placed dorsally, not ventrally. Second, the injury is incurred following a decompressive laminectomy. Third, the force (mass) does not persist following the initial impact.

A new SCI model in the rat has been devised in which a DeBakey aortic aneurysm vascular clamp has been modified to produce a ventral persisting mass (bone and/or soft tissue) without first performing a decompressive operation (i. e. laminectomy performed as part of the weight-drop technique). This model is capable of producing a wide spectrum of neurologic injury ranging from a minimal injury to a complete myelopathy.

The Tilt Board Technique was utilized for neurologic evaluation following the injury. The utilization of the above mentioned standardized vascular clamp technique in 24 surviving animals to date has resulted in 9 apparent complete myelopathies 13 apparent incomplete myelopathies and 2 animals without apparent injury. The percent loss of neurologic function (change in degrees of tilt via the Tilt Board Technique) lost in each group was 48%, 38%, and 12% respectively. Sagital post-morteum sections confirm a mass lesion located ventral to the spinal cord.

This experimental model appears to accurately reproduce the clinico-pathological situation seen in human SCI in which an apparent similar bony/soft tissue injury results in a variety of clinical manisfestations ranging from a complete myelopathy to no apparent injury with approximately a 20% mortality.

342.3 A PHYSIOLOGICAL ANALYSIS OF A HYBRID COMPUTER-CONTROLLED SPINAL CORD INJURY DEVICE. S.K. Somerson* and B.T. Stokes, Dept. Physiology, Ohio State Univ. Coll. of Med., Columbus, OH 43210

The time course of extracellular hypocalcia was studied in the rat spinal cord following the production of a mechanically predictable spinal cord injury. Animals were rigidly held by spinous process clamps and needles through the interspinous ligaments. The displacement (magnitude and duration) of the spinal cord during injury was regulated by a hybrid computer which used negative feedback circuitry to compare the preset displacement function to the actual displacement parameters. By allowing the delivered force to vary, a reproducible displacement profile could be achieved. The entire impact injury was over in app. 20 msec and the impactor withdrawn (< 4.0 msec) to a position (4.0 mm above the dural surface) that prevented subsequent contact of the animal with the device. The necessary force adjustments during injury also allowed an assessment of the variance in mechanical characteristics of the spinal compartment. Based on previous studies involving behavioral and histopathological assessments, three levels (light, intermediate and heavy) of displacement injury were produced. Impact duration for these levels were 14, 19 and 24 msec respectively; magnitudes ranged from 1.5-1.8 mm.

Calcium activities in the spinal cord of control animals showed little variation to electrode position or time of placement (mean +/- SEM = 1.08 +/- 0.01 mM). After injury, calcium activities at the injury site decreased to values below 0.1 mM and then recovered towards normal values over the next three hours. Incomplete recoveries occurred in the intermediate and heavy injury groups (0.72 +/-0.01 mM and 0.58 +/-0.01 mM respectively). Calcium activity in the light injury group recovered to normal levels by 3 hrs. Specific injury protocols therefore result in reproducible responses in the cellular microenvironment. In addition, since we have previously shown all animals in the light injury group have transient neurological and pathological defects, it is probable that acute hypocalcia transients are predictive of the subsequent outcome of acute spinal cord injury. (Supported by USPHS-10165 to The Ohio State University).

342.4 A BEHAVIORAL AND ANATOMICAL ANALYSIS OF SPINAL CORD INJURY PRODUCED BY A FEEDBACK CONTROLLED COMPUTERIZED IMPACTION DEVICE. Bresnahan, J.C., Todd, F.D.*, Noyes, D.H.*, and Beattie, M.S., Depts. of Anat., Physiol., Surgery (Div. Neurosurg.), and Neurosci. Res. Lab., The Ohio State University, Columbus, Ohio 43210.

The weight-drop method of Allen is still the most widely used experimental technique for spinal cord injury. In order to provide a reproducible experimental injury with immediate information on the actual mechanical impact properties, we have developed a feedback-controlled, electromagnetically-driven impactor. The device is sensitive to the characteristics of the injured tissue and allows continuous manipulation of force and displacement. The physical descriptors of each impact include cord surface displacement (cm) and force (kdyne), which yield impulse momentum (kdynes-sec), velocity (cm/sec), power (Mdyne-cm/sec), and work (kdyne/sec).

We have tested the device by delivering a wide range of impact intensities to the mid-thoracic spinal cord of rats. Impact descriptors were correlated with motor performance and lesion volume. Motor performance tests were administered preoperatively and over a 3 week post-injury period, and included open field walking (rated on a modified Tarlov scale), inclined plane test, and an agility test (grid-walking). The results showed significant correlations ($p < 0.01$) between lesion volume and all impact descriptors (rho = 0.81 to 0.93). Early in the recovery period, open-field walking and inclined plane performance correlated significantly ($p < 0.05$) with lesion volume and all impact descriptors. Later, performance on the agility test correlated ($p < 0.05$) with lesion volume and all impact parameters indicating that this test was most sensitive to residual neurological deficits.

We also tested the reliability of the device by setting it to produce a uniform time-displacement pulse, chosen from the prior experiment to yield a significant but partially reecoverable deficit. The results suggest that the descriptors of the actual impact are highly predictive of subsequent behavioral and histopathologic findings. Infrequent deviations from intended impact parameters are immediately noted.

Thus, the system provides three distinct advantages over previous methods: 1) a wide range of impact parameters; 2) a consistent and controllable lesion; and 33) immediate recognition of mechanical variability. (Supported by NIH Grant #NS 10165)

342.5 **A MOTOR EVOKED POTENTIAL AS AN INDICATOR OF RECOVERY IN CHRONIC SPINAL CORD INJURED CATS.** M. McCaffrey, W. J. Levy, and D. H. York. University of Missouri Health Sciences Center, Columbia, Missouri 65212

We have evaluated the predictive value for motor function of an evoked potential created by stimulating the motor cortex in spinal cord injured cats, both acute and chronic animals. This signal is derived from either direct or transcranial stimulation of the motor cortex, producing a descending activation of the pyramidal tracts which can be recorded in the peripheral nerves and produces EMG and movement responses. We have previously reported on the characteristics of this signal which is largely abolished by pyramidotomy and unaffected by red nucleus lesions. Its conduction velocity is approximately 100 meters per second and it travels in both dorsolateral and ventral cord. In a series of acute spinal cord injury cats, using a weight drop model, we determined the relative sensitivity of the motor evoked potential and the somatosensory evoked potential to cord injury. Adult conditioned cats were anesthetized under ketamine with arterial and venous lines placed and ventilated on a Harvard animal ventilator via a tracheostomy. Evoked potential monitoring was done with a Cadwell 7400. While the somatosensory evoked potential is frequently abolished by 200 gm cm of force, the motor evoked potential in the spinal cord is abolished at 100-150 gm cm of force. The motor evoked potential recorded in the peripheral nerve is much more sensitive to injury and is abolished by as little as 50 gm cm of force in some animals. In most cats, 75 to 100 gm cm of force will abolish the motor evoked potential in the peripheral nerve. It was observed at injury levels below those required to cause a temporary loss of the signal, the lesion produces an instability in the response. This results in a lessening of the frequency rate at which the signal will follow. At frequency rates below this, the signal fatigues, observed by sequential runs without pause recorded on the signal averager with both a progressive increase in latency and a decrease in amplitude evident. It was also observed that in some animals metabolic abnormalities, such as a metabolic acidosis, produce an instability in the signal from trial to trial with varying amplitudes and latencies. A series of chronic spinal cord injury to animals is now underway and we have observed that weight drop lesions in the 100-150 gm cm range abolish the motor evoked potential for an hour of post injury follow-up recording. They do not abolish the SEP, consistent with acute studies. However, these animals are paraplegic for a period of time ranging from days to weeks after injury. As they recover, the motor evoked potential recovers strength and consistently is stronger in the leg which is stronger in the ambulating cat. In some cases, the recovery of the motor evoked potential preceeds the recovery of function in the leg and can be used to predict the stronger leg. This suggests that the test is of clinical value in man since in humans recovery from spinal cord injury is much less effective than in the cat and lighter weight drop injuries would likely result in permanent damage. Therefore, the MEP is a better indicator of motor function than the SEP.

342.6 MECHANICAL PARAMETERS OF IMPACT CORRELATE WELL WITH OVERALL LOSS OF MYELINATED AXONS IN SPINAL CORD INJURY BUT NOT WITH DEMYELINATION, CALIBER SPECTRUM, OR RECOVERY OF FUNCTION. A.R. Blight, Depts. Neurosurgery and Physiology & Biophysics, New York Univ. Medical Center, 550 First Avenue, New York, NY 10016.

Contusion injury forms the most realistic controlled model of spinal trauma for studying pathophysiological mechanisms or potential treatment. Its usefulness has been limited, however, largely because of variability in the functional deficits that result from apparently 'standardized' mechanical impact, and because of the difficulty of measuring deficits or variability at sufficiently high resolution.

The characteristic pattern of axonal destruction and demyelination that follows contusion injury of the cat thoracic spinal cord was studied quantitatively by line-sampling in 1 µm plastic sections with the light microscope (see Neuroscience, 10: 521). Injuries were produced by a weight-drop with the vertebra below the impact (T9) stabilized by supports under the transverse processes. Using 2 groups of 10 animals, the effects of 2 levels of injury were examined: 10 or 13 g dropped 20 cm. The impact was delivered through a 5 mm dia teflon piston. Animals were kept for 3 months after injury, then fixed by perfusion.

The overall extent of axonal loss correlated well with the kinetic energy of the weight at impact, but only when expressed per unit cross sectional area of cord. Above 0.002 J $mm^{-2}$ there was practically no survival of axons at the lesion site. Between 0.0008 and 0.002 J $mm^{-2}$ the number of surviving axons varied between 100,000 and 2,000 with a linear correlation coefficient of -0.85.

Both caliber spectrum and myelin index distribution varied widely between animals, but neither variable correlated significantly with axon number or mechanical parameters. No recovery of hindlimb postural function was seen with less than 50,000 myelinated axons at the lesion center, but recovery of function in animals with more than 50,000 axons did not correlate well with axon number or injury mechanics.

Several precautions are necessary in interpreting data from contusion injuries: a) the size of the spinal cord must be taken into account; b) not all tissue pathology relates directly to the initial impact; c) tissue destruction is not distributed evenly and its extent is reflected poorly by testing the function of small subsets of axons. Given such precautions, contusion injury may provide the best possible combination of simplicity, realism and repeatability, but the phenomena themselves remain complex, intrinsically variable, and difficult to interpret without searching analysis. Supported by NS10164 and NS15590 from NINCDS and by the American Paralysis Association.

342.7 DEVELOPMENT OF CHANGES IN THE SPINAL CORD OF THE RAT AFTER CONTUSIVE INJURY. L.J. Noble and J.R. Wrathall. Dept. of Anatomy, Georgetown Univ. Sch. of Med., Washington, D.C. 20007.

We have previously described an experimental model of contusive injury in which groups of rats with mild, moderate, or severe functional deficit may be produced by a weight drop (WD) technique (EXP. NEUROL. 88,108-149,1985). Morphometric analyses of the epicenter (region of maximal disruption) at 4 weeks post-injury indicated significant correlations between the degree of functional deficit and areas of both the lesion and residual tissue. In particular residual white matter was highly correlated to functional deficit (r= -0.91) as measured by a combined behavioral score. We have now examined the development of these lesions with time after injury.

Rats were sacrificed at 15 min, 24 hrs, 1 wk, 4 wks, or 8 wks after injury. Spinal cord tissue was embedded in paraffin and transverse, serial sections were cut and stained for visualization at the light microscopic level. Sections representing the epicenter were measured using a digitizing pad and interfaced with an Apple IIe computer. The total cross-sectional area of the cord, the cavity, and the remaining gray and white matter were determined.

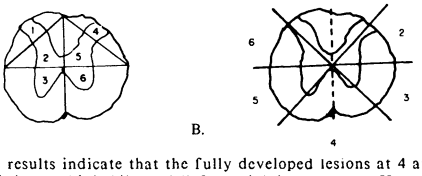
The time course of lesion development was generally similar for the different injury groups. 15 MIN AFTER INJURY. The gray matter was generally replaced by hemorrhage with the exception of the peripheral rims of the dorsal and (with lesser injuries) ventral horns. 24 HRS AFTER INJURY. A central cavity, containing cords of nonneuronal cells, typically replaced the ventral horn and most if not all the dorsal horn. The white matter contained variable numbers (depending on injury level) of axons and microcysts. Many of the axons appeared to be swollen. Demyelination was observed primarily in the dorsal columns and the paracentral white matter. 1 WK AFTER INJURY. The epicenter was characterized by a central cavity or core of granular, amorphous material. The residual white matter consisted of a complete or partial rim of tissue, exhibited regions of demyelination (most notably in the dorsal and dorsolateral white matter), and contained numerous microcysts and swollen axon cylinders. 4 AND 8 WKS AFTER INJURY. The epicenter consisted of a central cavity which contained primarily macrophages and was surrounded by a glial limitans. The remaining white matter exhibited areas of demyelination, and contained empty myelin sheathes, and microcysts. Many axon profiles were swollen and appeared granular and acidophilic. In a limited number of cases the epicenter was composed of only a meningeal framework enclosing a cavity.

Several significant trends were apparent from the quantitative analysis. First, the formation of a cavity was apparent between 15 min and 24 hrs and reached a maximum area at 1 wk after injury. Secondly, the total cross-sectional area of the cord became significantly less between 1 and 4 wks after injury. This was largely accounted for by a reduction in the area of the cavity. Third, the significant reduction in gray and white matter occurred between 24 hrs and 1 wk after injury. Thereafter, the areas of gray and white matter for each injury group remained constant. (Supported by NIH NINCDS contract NO1-NS-2-2310.)

342.8 SECTOR ANALYSIS OF SPINAL CORD LESIONS PRODUCED BY CONTUSIVE INJURY IN THE RAT. J.R. Wrathall and L.J. Noble, Dept. of Anatomy, Georgetown Univ., Washington, D.C. 20007

We have previously described a rat model of experimental spinal cord injury in which groups of animals with mild, moderate or severe functional deficits can be produced by dropping a 10 g weight 2.5, 5.0 or 17.5 cm respectively onto the exposed dura at the T8 level (EXP. NEUROL. 88:108-122, 1985). Morphometric analyses showed significant correlations between measures of the lesion epicenter (area of maximal damage) at 4 weeks after injury and the functional deficit as measured by behavioral tests (EXP. NEUROL. 88:135-149, 1985). Such analyses provide an overall view of the lesion but do not reflect important regional differences in the response of the spinal cord to experimental contusive injury. We have now examined different sectors of the spinal cord to investigate the loss of gray and white matter of the cord with time after injury.

Groups of rats were subjected to a 2.5, 5.0 and 17.5 cm weight drop injury and perfused with fixative at 15 min, 24 hr, 1 week, 4 weeks and 8 weeks after injury. Spinal cord tissue centering on the injury site was embedded in paraffin and serial transverse sections prepared. Sections representing the lesion epicenter were traced and subjected to quantitative analysis in terms of the total area, areas of recognizable gray and white matter, areas of hemorrhage and of cavitation in each sector of the cord. Sectors were defined by transparent overlays that divided the cord into 6 regions. For analysis of residual gray matter the 6 sectors included left and right dorsal peripheral (laminae I-IV), dorsal central (IV, V, VII, X) and ventral (VII-X) regions of the cord as shown in Fig 1A. For analysis of residual white matter radial sectors divided the cord into dorsal, left and right dorsal lateral and ventral lateral, and ventral regions as shown in Fig. 1B. Areas were traced on a digitizing pad and calculated using a computerized morphometry system (Bioquant).




The results indicate that the fully developed lesions at 4 and 8 weeks after injury exhibit bilateral (left vs right) symmetry. However, in all three injury groups more central than peripheral and more dorsal than ventral spinal cord tissue is lost. The time course studies indicate that not only is more central and dorsal tissue eventually lost but also that overt necrosis in these areas appears to occur at earlier times after injury. (Supported by NIH NINCDS contract NO1-NS-2-2310)

342.9 EFFECT OF SPINAL CORD INJURY ON THE "PARAVASCULAR" INFLUX OF HORSERADISH PEROXIDASE (HRP) FROM THE SUBARACHNOID SPACE. C.P. Barrett*, S.H. Deschner*, M.L. Rennels, J.P. Petrali*, E.J. Donati*, and L. Guth* Dept. of Anatomy, University of Maryland School of Medicine, Baltimore, MD 21201

After injection into the ventricles or subarachnoid space, HRP is rapidly distributed throughout the neuraxis via the perivascular spaces of large intraparenchymal blood vessels and the sleeve-like basal laminae around capillaries. This rapid "paravascular" distribution of tracer and its accumulation within the extracellular spaces defines a circulatory pathway by which the CSF equilibrates with the interstitial fluid compartment of the brain and spinal cord (Rennels et al., 326: 47, 1985). We examined the effect of localized spinal cord injury on this fluid pathway with the goal of determining whether the intrathecal route is useful for administering agents designed to promote healing of spinal cord lesions. Twelve adult female Wistar rats were anesthetized and, after laminectomy at T5, their spinal cords were crushed by extradural compression with a fine Dumont forceps. Six control rats were anesthetized and subjected to laminectomy without spinal crush. At selected intervals from 5 min to 3 days postoperatively, 4ul of a 6% solution of type VI HRP (Sigma) was injected into the lumbar cistern and 5 minutes later the animals were perfused intracardially with 1.5% glutaraldehyde-1.0% paraformaldehyde (in 0.1 M phosphate buffer, pH 7.4). Frozen sections were prepared and reacted for HRP activity using tetramethylbenzidine as chromogen. In both experimental and control rats, the tracer spread rapidly from lumbar to cervical levels and was distributed throughout undamaged regions of spinal parenchyma via the perivascular route. In regions of spinal cord crush, however, the tracer failed to enter the lesion via this route and, as a result, there was a zone of 2-3 segments in length that was free of HRP reactivity. These results indicate that access of CSF to the interstitium is diminished at sites of spinal injury. Such a decreased circulation of interstitial fluid could result not only in changes in extracellular pH, osmolarity, and ionic composition but might also produce secondary metabolic derangements that contribute to the extensive necrosis that accompanies spinal cord injury.

342.10 EFFECTS OF DORSAL BILATERAL RHIZOTOMY ON THE HIGH AFFINITY UPTAKE OF ($^3$H)-GLUTAMATE, -GABA AND -5-HT BY MYELIN-FREE SYNAPTOSOMAL PREPARATIONS FROM THE SPINAL CORD OF SPASTIC AND NONSPASTIC DOGS. P.V. Hall*, E. Chernet*, S. Shapiro*, C. Sartorius* and W.J. McBride. Depts. of Surgery & Psychiatry, Indiana Univ. School of Medicine, Indianapolis, IN 46223.

Previous studies from our laboratory (McBride et al., J. Neurochem. 42: 1625, 1984) indicated that the high affinity uptake of ($^3$H)-glutamate was greater in the spinal cord of spastic dogs than in the control group. One interpretation of the data is that the increase in uptake in the spastic group is due to axonal sprouting of the primary afferents and/or interneurons following degeneration of descending pathways. Using the high affinity uptake of glutamate as a marker, the present study was undertaken to test the hypothesis that (a) glutamate is the transmitter from some primary afferents, and (b) sprouting of glutamatergic primary afferents has occurred in the spastic animals. The myelin-free synaptosomal fraction, obtained by centrifugation procedures of the spinal cord of mongrel dogs, was used for the uptake studies. There were 4 groups of experimental animals: (a) sham-operated; (b) dorsal bilateral rhizotomy ($L_2$-$L_6$) for 1 week (DBR); (c) mid-thoracic crush for 4 or more weeks (spastic group); and (d) mid-thoracic crush for 4 or more weeks and DBR for one week (spastic + DBR). In addition to the high affinity uptake of 1 µM ($^3$H)-glutamate, the uptake of 1 µM ($^3$H)-GABA (an interneuronal marker) and 30 nM ($^3$H)-5-HT were also determined. The uptake of glutamate was 20% lower ($P < 0.05$) in the DBR group (245 + 35 pmol/min/mg protein, N=7) relative to the control group (299 + 33, N=7). The uptake of GABA was not different between the two groups (188 + 15 pmol/min/mg protein for sham vs. 178 + 21 for DBR). Surprisingly, there was a 45% decrease in the uptake of 5-HT in the DBR group (0.57 + 0.10 pmol/min/mg protein, N=6) compared to the control values (1.04 + 0.23, N=6). As expected, midthoracic crush of the spinal cord produced a 97% decrease in the uptake of 5-HT, indicating a loss of descending serotonergic fibers. One week after DBR treatment of the spastic dogs, there was a significant ($P < 0.005$) 25% reduction in the uptake of both glutamate and GABA. The 20% lower uptake of glutamate in the DBR group relative to sham operated is consistent with the idea that a portion of the primary afferents may use glutamate as a transmitter. Furthermore, the finding that DBR treatment of spastic dogs reduced the elevated glutamate uptake by 25% may indicate some sprouting of the primary afferents has occurred in the spastic dogs. However, the reduction in the uptake of GABA with DBR treatment of the spastic group and of 5-HT with DBR treatment of normal dogs is difficult to explain.

## MOTIVATION AND EMOTIONS

343.1 PROPOSED ANXIOGENIC ENDOCOID: MODULATION BY GABA AND BENZODIAZEPINE SYSTEMS. C.M. Harris and H. Lal. Dept. of Pharmacol., Tex. Coll. Osteopath. Med., Fort Worth, TX 76107

Chloride (Cl) conductance is mediated by an ionophore located in a supramolecular complex with receptors for GABA, benzodiazepines (BZ), pentobarbital (PB), picrotoxin, and other still unidentified ligands. BZ, PB and GABAergic agents increase Cl conductance and reduce anxiety (Lal et al., Neuropharmacol. 19:785, 1980). If there is an endogenous substance (endocoid) which produces anxiety by inhibiting Cl conductance, its action should be unmasked by removal of the endogenous influences which stimulate the GABA and BZ systems. Towards testing this hypothesis, anxiogenic properties of antagonists for GABA and for BZ were tested singly and in combination in an animal model for anxiety. This model provides a rate-independent assay for anxiogenic drugs (Lal and Emmett-Oglesby, Neuropharmacol. 22:1423, 1983). Rats were trained in a food-reinforced operant task to select 1 of 2 levers after injection of the anxiogenic drug pentylenetetrazol (PTZ), and the other lever after saline. These rats selected the PTZ-lever after PTZ and the other lever after saline, diazepam (DZ) or PB. PTZ-lever selection was blocked by pretreatment with DZ or PB. Subconvulsant doses of GABA antagonists (bicuculline, 2.5 mg/kg, or picrotoxinin, 0.32 - 0.64 mg/kg) and of a GABA synthesis inhibitor (isoniazid, INH, 100 - 200 mg/kg) failed to mimic PTZ. Similarly, BZ-receptor blockers (RO15-1788, 2.5 - 40 mg/kg, and CGS 8216, 10 - 40 mg/kg) also failed to mimic PTZ. However a marked PTZ-like effect was observed after combinations of a subconvulsant dose of INH (200 mg/kg) with low doses of the BZ antagonists (RO15-1788, 0.64 - 10 mg/kg, or CGS 8216, 2.5 mg/kg). The PTZ-like stimulus produced by the combination of RO15-1788 and isoniazid was blocked by PB, 5 mg/kg. These data support the hypothesis that there is an anxiogenic endocoid, and that its anxiogenic effect is antagonized by ligands for the BZ, PB and GABA receptors. The BZ and GABA systems appear to act in a concerted manner such that, if the function of one system is compromised, the other system compensates for and/or facillitates the function of the compromised system. It is unlikely that the site of action of this endocoid is the BZ receptor because an anxiogenic effect was detected when the BZ receptors were blocked. The site of action for this anxiogenic endocoid may be the same as that for PB because PB blocked the anxiogenic effect. Both PB and the endocoid may act directly at the Cl ionophore, with the endocoid blocking, and the PB increasing Cl conductance. However, another site cannot be ruled out.

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343.2 EFFECTS OF THE BETA-CARBOLINE, FG 7142, ON THE SOCIAL BEHAVIOR OF MALE RATS IN A LIVING CAGE. C.H.M. Beck[1] and S.J. Cooper[2], [1]Dept. of Psychology, University of Alberta, Edmonton, Alberta, Canada T6G 2E1, [2]Dept. of Psychology, University of Birmingham, Birmingham B15 2TT, U.K.

The benzodiazepine inverse agonist FG 7142 (N'methyl-ß-carboline-3-carboxamide) decreased the time during which a pair of male rats interacted with each other in an arena (File, S.E. & Pellow, S., Arch. Internat. Pharm. Ther., 271:198, 1984). The purpose of the present study was to describe the effects, and their time-courses, of FG 7142 on social behavior in more detail. Four 6-min dyadic social encounters in a familiar living cage were observed in a paradigm in which one member of a pair of rats was injected. The behavior of the injected animal was coded on a microprocessor into exhaustive and mutually exclusive behavior categories including approaching, avoiding, and fighting with the other rat, locomoting, sniffing the cage, self-grooming and immobility. The four treatments were i.p. injections of vehicle (distilled water + Tween 80), and FG 7142 at 2.5, 5.0 and 10.0 mg/kg, respectively. N = 8 pairs of adult male hooded rats per group (body weight range: 178-262 g). All injections were administered 2 min before the start of the first observation trial; the observer was blind to injection conditions.

Compared to the effects of vehicle injection alone, FG 7142 decreased aggressive behavior but did not alter the total level of social interaction. This was because FG 7142 induced compensating increases in approaching and avoiding the other animal. The FG 7142 effects on these social behaviors were most apparent in the first two trials. All were dose dependent. Locomotion declined marginally and immobility increased in FG 7142-injected rats. Twitches and convulsions were seen in 3 of the 10 rats given 10 mg/kg FG 7142.

The rats in the foregoing study had been housed in pairs prior to testing. In a second experiment in which rats housed in isolation were used, vehicle-injected animals spent twice as much time in aggression as did the control animals in the pair-housed study. In spite of the increase in level of aggression, FG 7142 at 5.0 mg/kg again significantly decreased aggression. Thus our studies demonstrate that the inhibitory effect of FG 7142 on social behavior in male rats may be specifically related to aggression in situations which permit the expression of agonistic displays.

FG 7142 was generously supplied courtesy of Dr. E.N. Petersen, A/S Ferrosan, Copenhagen.

343.3 HYPOTHALAMIC TEMPERATURES OF RATS BEHAVIORALLY THERMOREGULATING IN 2450-MHz MICROWAVE FIELDS. D.M. Levinson (Univ. Missouri-K.C., 64110; & VA Med Ctr, K.C., MO), D.W. Riffle and D.R. Justesen (VA Med Ctr, Kansas City, MO 64128).

Four female Long-Evans rats, each with a chronic cannula implanted in the brain (target area: preoptic hypothalamus), were observed for performance of a lever-depression task in which they controlled either a 2450-MHz multipath microwave field at various dose rates (30, 60, or 90 mW/g) and at a constant ambient temperature (25.3°C); or the 2450-MHz field at a constant dose rate (60 mW/g) and at various ambient temperatures (17, 25, and 33°C). During daily 30-min. sessions (2 under each condition), each depression of the lever extinguished the field; which remained extinguished while the lever was depressed. A photic cue was presented in temporal synchrony with the field. Hypothalamic temperature was measured continuously by a Vitek thermometer with a field non-perturbing probe, which was acutely inserted into the cannula during each 30-min. session. Pre- and post-session colonic temperatures were measured by a Bailey thermometer.

Final means of hypothalamic and colonic temperature did not differ reliably, and under most conditions they ranged between 38.5°C and 39.5°C. (Under 17°C conditions, final temperatures ranged between 37.7°C and 40.1°C.) Means of hypothalamic temperatures typically rose 0.5 - 1.5°C above pre-exposure baselines within the initial 5 min. of a session, after which they gradually and reliably fell during the remainder of the session, for both varying dose rates [$F$ (9,27) = 13.01, $p$ <.001] and ambient temperatures [$F$ (9,27) = 5.50, $p$ <.001]. Hypothalamic temperatures did not reliably differ as a function of either dose rate or ambient temperature [both $F$s(2,6)<1.00]; however, there was a reliable interaction between minutes of sessions and ambient temperatures [$F$ (18,54) = 2.74, $p$ <.01]. This interaction is reflective of gradually declining temperatures following the initial rise for the 17°C and 25°C conditions, as opposed to no decline in temperatures following the initial rise for the 33°C condition. Times in the field were highly and negatively correlated with dose rates and ambient temperatures ($r$s (1) = -.94 and -.99 respectively], decreasing as these increased; further, times in the field differed reliably both as a function of dose rate [$F$ (2,6) = 82.22, $p$ <.001] and ambient temperature [$F$ (2,6) = 7.91, $p$ <.05]. These data indicate that rats exposed to fairly intense microwave fields will behave in a manner which enables them to maintain relatively consistant hypothalamic temperatures; the data also indicate that at higher ambient temperatures the animals' ability to thermoregulate behaviorally may become impaired, possibly because of thermal overload.

343.4 INVOLVEMENT OF THE DOPAMINERGIC PROJECTION FROM VENTRAL TEGMENTAL AREA TO NUCLEUS ACCUMBENS IN THE MOTIVATION UNDERLYING TWO-WAY AVOIDANCE IN THE RAT. W. J. Wilson and E. G. Baeske*. Department of Psychological Sciences, Indiana University - Purdue University at Fort Wayne, Fort Wayne, IN 46805.

Lesions of the nucleus accumbens (1,2) or the ventral tegmental area (VTA) (3) enhance two-way avoidance responding in rats. Izquierdo and his colleagues (4) have identified three independent factors that motivate shuttle responding in the signalled two-way avoidance paradigm. They are a "drive" state (D), resulting from nonassociative effects of the shock, a "pairing" factor (P) due to the temporal contiguity between signal and shock, and a "contingency" factor (C) that arises because a response during the signal effectively avoids the shock. Schutz and Izquierdo (2) have shown that the enhancement of avoidance by accumbens lesions is due to an increase in D. We are examining VTA lesioned rats to determine whether the enhancement of avoidance behavior is mediated by a similar increase in D, or instead by a change in P or C. If both lesions increase D, then the VTA-accumbens system must normally inhibit generalized drive.

Rats receive either bilateral electrolytic lesions of the VTA or sham lesions. They are then placed in a shuttle box and exposed to one of four behavioral paradigms in which 5 sec tones and 1.5 mA scrambled ac shocks are presented either with or without temporal contiguity, and with or without a contingency between responding during the tone and shock avoidance. The paradigms are:

D) no contiguity, no contingency; tones and shocks occur randomly, shuttle does not avoid shock.

DP) contiguity, but no contingency; tones always precede shock, but a shuttle is ineffective.

DC) contingency, but no contiguity; tones and shocks presented pseudo-randomly, shuttle during tone avoids the next shock.

DPC) contiguity and contingency; tones always precede shock, shuttle during tone avoids the next shock.

According to Izquierdo's analysis, a comparison of the shuttle responses to the tone in each of the four conditions by the VTA and sham lesioned rats will allow the determination of any differences in D, P, or C between the two groups. Such an analysis will indicate any similarities in the motivational involvement of the accumbens and the VTA.

1. Lorens et al. JCPP, 1970, 73, 284 - 290.
2. Schutz & Izquierdo. Physiol Behav, 1979, 23, 97 - 105.
3. LeMoal et al. Physiol Behav, 1969, 4, 567 - 573.
4. Izquierdo & Elisabetsky, in M. A. B. Brazier (Ed.), Brain mechanisms in learning and memory: From the single neuron to man. Raven: New York, 1979.
5. Izquierdo & Cavalheiro. Psychopharmacology, 1976, 49, 145-157.

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343.5 **AUGMENTING/REDUCING OF CAT VISUAL EVOKED POTENTIALS AND INDIVIDUAL DIFFERENCES IN BEHAVIOR.** P. M. Saxton, J. Siegel and J. H. Lukas*[1]. Inst. for Neuroscience, Univ. of Delaware, Newark, DE, 19716.

The change in amplitude of the visual evoked potential (VEP) to an intensity series of light flashes varies between cats. Individuals showing increasing amplitudes to increasing intensities are termed augmenters; reducers show decreasing amplitudes at the highest intensities. A/R slopes correlate with ratings of cat behavior to novel or frightening stimuli. This experiment examines if cortical A/R is predictive also of an inhibitory capacity to control behavior under conditions of stress.

Eight cats were implanted with electrodes over primary visual cortex and VEPs were collected. Behavioral ratings were correlated with slopes of components NI and PI-NI to medium and high flash intensity ranges. Responses to novel stimuli indicated that augmenting cats were more active (r=0.55 to 0.72 for NI and PI-NI averaged slope at medium intensity range) and explorative (.52). Reducers vocalized more, were difficult to handle and aggressive toward other cats. Cats were trained to bar press for food reward in a cued two bar situation at a fixed interval (FI) and then in a differential reinforcement of low rate of response (DRL) paradigm. The disruptive effects of loud noise bursts during the FI and DRL performance and increasing task difficulty in the two bar DRL were tested. Latency to learn, rate of bar pressing, number and type of errors and efficiency were recorded for each 30 minute session.

When correlated with bar press learning PI-NI slopes at the high intensity range were more predictive. Reducers took longer to habituate to the situation (-.81) and to learn to bar press (-.4), and more days to reach FI criterion (-.49). Overall bar pressing ability correlated significantly (.71) with the high range PI-NI slope, indicating that augmenters, being more active and explorative, learned to bar press quickly and were better at it. A/R slope also correlated (.78) with extra presses in the two bar situation which is consistent with augmenter's exploratory behavior and faster learning.

In contrast to the FI task, in the DRL correlations were stronger with the NI slopes at the medium intensity range. In this inhibitory task augmenters took longer to learn (.76) and had less change in efficiency with noise. Augmenters did so poorly on this inhibitory task that they consequently showed less change when the task was made more difficult or stressful. In ranking the cat's overall control in the DRL task augmenters showed least control (.72).

The slope of PI-NI at high intensities correlates with spontaneous behaviors and learning of a FI task, while the A/R slope of the NI component at a medium intensity range is more predictive of inhibitory control required on a DRL task. Augmenters are active and responsive, learn the FI task faster, bar press vigorously and hence work with maximum efficiency. Reducers, on the other hand, show not only cortical inhibition but an enhanced capacity for learning and performing on an inhibitory task.

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343.6 MULTIPLE UNIT ACTIVITY IN RESPONSE TO MOTIVATIONAL AND VISUAL STIMULATION. M. Morales* and J.J. Keene* (SPON: C. Zuazaga). Dept. of Physiology, Lab. of Neurobiology, Univ. Puerto Rico, San Juan, 00901.

Neural responses to motivational and sensory stimuli were analyzed using the multiple unit activity recording technique. Data was collected from 6 cat cerveau isole preparations. The data contained samples from cortex, thalamus, internal capsule and caudate. Motivational stimuli were applied to the medial forebrain bundle (M) and the mesencephalic reticular formation (R), which were rewarding and aversive inputs respectively. The sensory stimulus was visual and consisted of a pair of incandescent bulbs placed in front of the cat's eyes. The motivational and sensory stimuli were delivered separately and in combination in random order. Extracellular recordings were taken for an initial period of a 0.5 second, (during the intervals between the stimulus train of pulses), and during a prolonged period for 4.5 seconds.

Specific interactions between the motivational and sensory stimuli were found in response to particular stimulus combinations. In the cingulate cortex the M stimulus elicited an inhibitory response while the R stimulus was excitatory. When both stimuli were delivered together there was a cancellation of these effects. These opposite responses reflect the opposite motivational significance of the stimuli. In the presence of light, however, the M-elicited inhibition and the R- elicited excitation were abolished. On the other hand, in the ventral anterior- ventral lateral nuclei (VA-VL) of the thalamus the R stimuli elicited pronounced inhibition while M stimulation alone elicited no effect. However, when the M and R stimuli were delivered together the R-elicited inhibition was abolished. This gating effect persisted in the presence of the light stimulus.

While the opposite responses observed in the cingulate cortex and M gating of R-elicited inhibition observed in the VA-VL nuclei may both be neural representations of the rewarding and aversive properties of the intracranial stimuli, it is interesting that only the differential responses in VA-VL persist in the presence of the light stimulus. These effects were localized in these two structures. In addition to these differential responses to M and R stimuli, similar responses were found in the somatosensory cortex, visual cortex, posterior lateral thalamus and caudate. Thus the interactions observed were highly localized in specific anatomical structures. (Partially supported by NIH Grant RR-08102).

343.7 INHIBITION OF CRICKET KILLING BY LATERAL HABENULAR LESIONS.
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Electrolytic lesions were used to study the role of the lateral habenula in cricket and mouse killing by rats. Long-Evans hooded rats which spontaneously killed crickets but not mice were used as subjects. Behavioral tests included cricket killing, mouse killing, reactivity, activity, locomotion, food and water intake, food spillage, and weight. The experiment consisted of a mixed within and between subject design. The between subject variable was treatment condition: bilateral habenular lesion (.8 ma, 30 sec.) (N=10), sham lesion (N=16), or unoperated control (N=10). The within subject variable was repeated tests with one pretest and two posttests for each of the measures, with the exception of food and water intake, food spillage, and weight which were measured daily. Cricket killing was tested during the sixth hour of the light cycle and mouse-killing during the sixth hour of the dark cycle one day prior to surgery and on days 4 and 8 postoperatively. Reactivity testing occurred 3 days prior to surgery and on days 3 and 7 postoperatively. Activity and locomotor ability testing occurred 2 days prior to surgery and on days 5 and nine post-operatively. A multivariate repeated measures analysis of variance was performed on all data. A significant treatment by time interaction ($p = .007$) was found for cricket killing. Cricket killing was significantly inhibited for the lesioned group of both posttests. A significant treatment by time interaction ($p = .002$) for food intake was also found for the lesioned group with food intake significantly decreased immediately following surgery but returning to baseline by nine days postoperatively. No other measure was significantly affected by the lesions. This inhibition of cricket killing by habenular lesions is hypothesized to result from the release of the mesencephalic raphe from inhibition resulting in elevated activity of serotonergic neurons.

343.8 INTRASPECIFIC AGGRESSION IN CATS AFTER DUAL ELECTRICAL STIMULATION OF SEPTUM AND HYPOTHALAMUS.
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The working hypothesis that intraspecific aggressive behavior is related to different neural fields in functional interdependence was analyzed by single and dual brain stimulation in free cats. It is now well known that septum selectively suppresses interspecific aggression elicited by lateral hypothalamical stimulation (Siegel, A. and Skog, D., Brain Research,23: 371-380, 1970). The present study analyzes whether the concurrently electrical stimulation of both structures might also influence intraspecific aggression. It is an extension of previous observation that simultaneous stimulation of other brain areas also modulates agonistic or intraspecific aggression (Ramirez, J.M. et al. Aggressive Behavior,9:104, 1983).

For this purpose cats were prepared with 30 to 50 stereotaxically implanted needle guides in the skull through which roving electrodes were lowered at 0.5 mm steps. After repeated functional testing, permanent electrodes were implanted in those sites whitin the ventromedial hypothalamus capable of eliciting some fragments of agonistic behavior and in those others within the septum capable of significantly modulating the mentioned behavioral fragments.

Single VMHT stimulation elicited many different behavioral effects: overt autonomic components, such as pupillary dilation, deep breathing, piloerection and salivating, and visible motor signs, such as arching of the back, baring of teeth and hissing. Among the different components observed, hissing was selected for the dual stimulation analysis. Concurrent stimulation of VMHT and septum showed their modulation of intraspecific aggression through a functional interrelation of both structures. Stimulation of much of septal areas -both, ipsilateral and contralateral to VMHT stimulating points- resulted in significantly facilitation of hypothalamically-elicited behavior.

Results suggest that these both brain structures, as many others, are functionally interrelated and coordinate spatial and temporal appearance of many agonistic components. Next step should be to determine which are the structures and pathways involved into the regulation of intraspecific aggression and to clarify their mechanisms of action.

343.9 14C-DEOXYGLUCOSE IDENTIFICATION OF EFFERENT PATHWAYS OF ATTACK INDUCED BY STIMULATION OF RAT HYPOTHALAMUS. W.W. Roberts. Department of Psychology, University of Minnesota, Minneapolis, MN 55455.

Electrodes were chronically implanted in the anterior ventral region of the lateral hypothalamus of male rats, where electrical stimulation induces vigorous biting/jumping attack on mice and rats. After tests for attack, male sexual behavior, eating, drinking and gnawing, rats were selected for an attack group that displayed strong attack during stimulation and for a stimulated control group that evidenced no attack but displayed other behaviors similar to those accompanying attack in the first group (exploratory behavior, upward escape jumping, turning, etc.). 14C-deoxyglucose was administered via a chronic caval catheter while the rats were anesthetized, and intermittent stimulation was administered for 45 minutes. After perfusion with 3.3% formalin, the brains were removed and sectioned in a cryostat, and the dried sections on coverslips were exposed to x-ray film. A diagrammatic analysis was made of areas darkened unilaterally in the attack group, followed by a quantitative densitometric comparison of the attack and stimulated control groups.

Although a considerable number of ascending, lateral, dorsal, and descending projections were evident in the autoradiographs, one descending system was most strongly associated with attack: the bilateral ventral supraoptic commissural path that follows the optic tract to the midbrain peripeduncular area and thence 1) medially through the posterior ventral thalamus to the periventricular gray, and 2) posteriorly to the vicinity of the cuneiform nucleus. A considerably weaker attack-related projection was located in the periventricular and central gray.

A separate correlational analysis of upward escape-like jumping, which was similarly represented in both groups and therefore orthogonal to the attack response, disclosed a close association in the combined groups with a number of ipsilateral medial structures, including an anterior projection through the medial preoptic area to the lateral septal area, a dorsal extension to the thalamic periventricular/parataenial region, and a posterior projection through the posterior hypothalamus to the dorsal premamillary nucleus and dorsal periventricular gray.

343.10 ANATOMY OF AFFECTIVE VOCALIZATIONS IN CHICKS.
L. Normansell* P. Bishop* & J. Panksepp. (SPON: F. DeEskinazi). Department of Psychology, Bowling Green State University,Bowling Green, OH 43403.

Areas associated with the production of social separation-induced distress vocalizations (DVs) have been extensively studied in the domestic chick. Distinguishing systems directly controlling DV output from neural circuits which act indirectly by modulating emotional response to social isolation is more difficult. We attempted such a discrimination using brain lesioning techniques aimed for circuits from which DVs can be elicited with electrical stimulation.

Three day old domestic chicks received bilateral lesions of either the ventral archistriatum (Av), the medial intercollicular area (MICo), dorsomedial thalamus (DMT), or Area C. Number of DVs emitted during 10 min of social isolation was assessed 2 days after surgery. MICo lesions reduced DVs by 95.4%, DMT lesions by 68.8%, and Area C and Av by 51.7 and 31.2, respectively. Immediately after isolation testing, like-lesioned birds were paired in an open field for 3 min. Both Av and MICo lesioned groups showed a complete cessation of DVs, while DMT and Area C lesioned animals showed a 73.8 and 60.9% reduction, respectively. The following day vocalization rates were assessed in a cold stress condition. Animals with DMT lesions increased 122.6% from their pre-cold baseline, suggesting this area, as well as the Av, participates in the emotional modulation of DVs. Lesions in both ICo and Area C produced reductions in DVs in all experimental conditions, indicative of a probable disruption of the CNS areas controlling the more generalized expression of DVs.

In a follow-up study, animals lesioned in the MICo were injected intracerebroventricularly with 1 ug curare, reported earlier to activate vocalization circuitry (Panksepp, et al, Soc Neurosci Abstr 9: 979, 1983). Animals whose rates of DVs were reduced 53% following surgery, returned to control levels following injection. Animals in which lesions totally eliminated DVs were apparently unresponsive to the vocalization promoting effects of curare, although flight behaviors remained intact.

343.11 2-DEOXYGLUCOSE STUDIES OF DEPRESSED BEHAVIOR IN RATS.
S. Caldecott-Hazard, D. Slabaugh*, R. Ackermann, J. Mazziotta, M. Phelps.
Nuclear Medicine and Biophysics, UCLA Medical School, L.A., CA. 90024

Human studies using positron emission tomography (PET) are limited by an inability to use some invasive techniques and experimental drugs. Therefore we are developing 3 rat models to study mechanisms underlying changes in local cerebral glucose utilization, identified by PET in depressive disorders. Our models were induced by multiple injections of alpha-methyl-para-tyrosine over a 12 hour period (AMPT) (Rech et al, J PET, 153:412 1966), withdrawal from amphetamine administered by subcutaneous pellets for 4 days (AmpWD) (Ellison et al, Science, 201:276, 1978), or 3 weeks of physical and psychological stressors (Katz et al, Neurosci & Biobehav. Rev, 5:259, 1981). Two symptoms of depression in humans, motoric retardation and weight loss, were measured in rats. Locomotor retardation was studied using an open-field apparatus. The number of squares crossed per minute was significantly reduced in each of the 3 models compared to controls. The antidepressant, tranylcypromine reversed this depression under AMPT and AmpWD conditions but by itself did not affect behavior. Weight loss during the period of model induction was also significant in each of the 3 models as compared to controls.

Glucose metabolism was studied using 14-C-2-deoxy-glucose (2DG) quantitative autoradiography. Metabolism was decreased globally by 26% in the stress model (N=6, $p<.05$), 40% in the AMPT model (N=4), and 22% in the AmpWD model (N=3). Also, the medial prefrontal cortex, anterior ventral thalamic nucleus, ventral hippocampus, and dorsal tegmental nucleus of Gudden showed bilateral reductions in metabolism in each of the 3 models. The lateral habenula showed bilaterally increased metabolism in all models. Tranylcypromine, which by itself had no effect on metabolism in controls, reversed the habenula's increased metabolism under AMPT, concommitant with reversal of depressive behavior.

These results indicate that both global changes and specific limbic structures may mediate depressed behavior in rats. These data parallel findings in bipolar depressed patients of 29% reductions in supratentorial global metabolism, compared to normals, with specific decreases in frontal cortex and thalamus (Baxter et al in press).

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343.12 TAIL PINCH-INDUCED BEHAVIORS IN DORSAL RHIZOTOMIZED RATS.
T.R. Gallub*, D. Berman & E. Schneider*. Dept. of Psychology, Queens College, CUNY, Flushing, NY 11367.

Animals with section of the dorsal roots innervating a limb tend to self-mutilate the deafferented extremity. This phenomenon has been interpreted as a response either to putative dysesthesias or to the animal's perception of the limb as a foreign object. In an effort to clarify this issue, dorsal rhizotomized (DR) rats were subjected to intermittent tail pinch. Tail pinch in intact rats results in chewing and gnawing, typically directed at food or other chewable objects (Antelman & Szechtman, Science, 189: 731, 1975). It was reasoned that, if an insensate limb were perceived as an extra-personal inanimate object, it would become a target for tail pinch-induced chewing.

Male Long-Evans rats were subjected to dorsal rhizotomy of the left forelimb (C6-T1, n=5) or hindlimb (L1-L6, n=4; T13-L6, n=1). Two and a half to twelve months later, food-sated operated and unoperated control animals (n=5) were tested with tail pinch with and without food, but with no other chewable stimuli available. In the presence of food, but without tail pinch, no animal showed chewing behavior. During tail pinch in the absence of food, intact animals showed increased restlessness and irritability, but no chewing or licking directed to any body part; in the presence of food, they showed energetic licking and chewing of food pellets.

Four of the five forelimb DR animals and all of the five hindlimb DR animals subjected to tail pinch showed licking of the deafferented limb when no food was present, sometimes actually drawing blood. Even when food was present, they responded to tail pinch with licking of the affected limb, as well as licking and chewing of the food.

In a preliminary attempt to address the issue of whether a dysesthesic state underlies post-dorsal rhizotomy self-mutilation, intact and DR rats were injected with formalin subcu. This procedure elicited licking of injected intact limbs, but not of injected deafferented limbs. When tail pinch was applied to intact animals 20 and 40 min after injection, all licking ceased and was replaced by agitated behaviors, such as backing up, licking the bowl, and attempts at escape.

These results are not incompatible with the hypothesis that DR rats perceive their insensate limbs as foreign objects. However, the extremely short latency in some animals from the onset of tail pinch to the start of vigorous licking of the affected limb, even in the presence of food, suggests a more active process. It is improbable that tail pinch-induced licking of the DR limb is related to nociception as tail pinch reduced, rather than increased, limb licking after formalin injection.

## MOTIVATION AND EMOTION: REWARD SYSTEMS

344.1 KAPPA RECEPTORS MEDIATE THE AVERSIVE MOTIVATIONAL EFFECTS OF OPIATES. A. BECHARA* and D. van der Kooy. Neurobiology Research Group. Dept. of Anatomy, University of Toronto, Tornto, Canada M5S 1A8

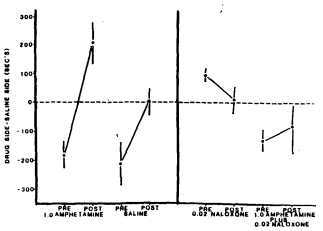
Endogenous and exogenous opioids produce positive reinforcing effects through an action on central nervous system opiate receptors and aversive effects through an action on peripheral opiate receptors. We now report that in addition to the anatomical specificity of the motivational effects of opiates, there may be a pharmacological specificity as well. Employing a place conditioning paradigm, separate groups of drug naive rats were administered various subcutaneous or intraperitoneal doses of the specific Kappa receptor agonist U50,488 (.005-16 mg/kg) or of morphine (.005-1.25 mg/kg). Regardless of the route of administration, increasing doses of morphine produced conditioned place preferences, whereas a wide range of doses of U50,488 produced conditioned place aversions. Only a low dose of morphine (.05 mg/kg) i.p. but not s.c., produced significant place aversions, suggesting a local gut effect, possibly on Kappa receptors in the gut. Although morphine is about 8 times more potent than U50,488 in producing analgesia, the lowest dose of U50,488 that produced aversions was 5 times lower than the low dose of morphine that produced aversions. Even at very high doses, U50,488 never produced the conditioned place preferences seen with morphine. Vagotomy blocked the aversive properties of both U50,488 and morphine. The results suggest that peripheral Kappa receptors mediate the aversive effects of opiates, whereas central mu receptors mediate the positive reinforcing effects. The motivational effects produced by selective stimulation of central Kappa receptors remain to be investigated.

344.2 NALOXONE SUPPRESSES SELF-STIMULATION BY CENTRAL BLOCKADE OF REINFORCEMENT. J. D. Belluzzi, K. A. Trujillo, C. D. Sun*, and L. Stein. Department of Pharmacology, College of Medicine, University of California at Irvine, Irvine, CA 92717.

Opiate receptor antagonists, such as naloxone (NAL) and naltrexone (NTX), suppress self-stimulation (SS) of enkephalin-rich brain regions following peripheral administration. According to the endorphin theory of reinforcement, these antagonists suppress SS by blocking central opiate receptors, thereby preventing the reinforcing action of stimulation-released endogenous opioids (Belluzzi and Stein, 1977). However, since SS can be suppressed by effects unrelated to reinforcement, others have suggested that these drugs may produce nonspecific performance deficits. The present studies tested whether opiate antagonists interfere with reinforcement or performance in SS. Since the reinforcing effects of brain stimulation are centrally mediated, one would expect that the suppressant effects of opiate antagonists would also involve central processes. Rats were trained to lever-press for nucleus accumbens brain stimulation in 1-hr daily sessions. When stable baseline rates were obtained, NAL and NTX were compared to their quaternary derivatives, naloxone methobromide (BrNAL) and naltrexone methobromide (BrNTX), for potency to suppress SS. BrNAL and BrNTX do not cross the blood-brain barrier and therefore block only peripheral opiate receptors. NAL (0.2, 2.0, and 20 mg/kg s.c.) caused significant suppression of SS (83.0, 61.7, and 35.0 mean % of saline control, respectively), whereas even the highest dose of BrNAL examined (20 mg/kg s.c.) had no effect on this behavior (95.4 % of control). Similarly, NTX (20 mg/kg s.c.) produced a strong suppression (42.6 % of control) while BrNTX showed no effects (95.7 % of control). These findings suggest that opiate antagonists suppress SS by blocking opiate receptors in the brain. To determine if opiate antagonists suppress SS by interfering with the ability of the animals to respond, the effects of NAL were compared on lever-pressing and nose-poking for nucleus accumbens SS. Nose-poking is a simple response requiring little motor output, whereas lever-pressing is more difficult. If opiate antagonists act by interfering with motor capacity, then nose-poking should be relatively unaffected by NAL. On the other hand, if these drugs act by blocking reinforcement, then nose-poking and lever-pressing should be equally suppressed (Leibman, 1983). NAL (0.2, 2.0, and 20 mg/kg s.c.) suppressed nose-poking and lever-pressing for SS equally (65.3 vs. 66.3; 38.6 vs. 35.8; 29.5 vs. 21.2 mean % of saline control, respectively). These results suggest that opiate antagonists suppress SS by interfering with reinforcement rather than motor output. Our findings support previous work demonstrating that NAL and NTX produce an extinction-like response decrement pattern in SS (Trujillo, Belluzzi and Stein, 1984) and are consistent with the suggestion that endogenous opioids mediate brain stimulation reward. (Supported by AFOSR grant 84-0325)

344.3 NALOXONE BLOCKADE OF AMPHETAMINE REWARD IN PLACE PREFERENCE CONDITIONING. K. A. Trujillo, J. D. Belluzzi, P. R. Tabrizi*, and L. Stein. Department of Pharmacology, University of California, Irvine, CA 92717.

The conditioned place preference (CPP) paradigm was used to study possible interactions between endogenous opioids and catecholamines in reinforcement. These neurotransmitters have been suggested to serve as putative "reinforcement transmitters" in the brain. In addition, studies suggest that the endogenous opioids and catecholamines may interact in reinforcement. In the present studies amphetamine (AMPH), which potentiates the release of catecholamines, and naloxone (NAL), a potent and selective opiate receptor antagonist, were examined alone and in combination in CPP. Two identical shuttle boxes were used in which two compartments are distinguished by color, odor, and texture. Initial compartment preferences were determined by measuring the time spent in each compartment for three baseline days. Rats were then conditioned by pairing one compartment with drug and the other with saline over eight 30 minute sessions. The reinforcing or aversive properties of a drug were determined in post-conditioning tests by measuring the change in compartment preference from baseline. Reinforcing effects were assessed by pairing drug injections with the initially non-preferred compartment, while aversive effects were assessed by pairing drug with the initially preferred compartment. As observed in previous studies, AMPH (1.0 mg/kg, sc) shifted place preference to the compartment associated with drug, demonstrating AMPH's reinforcing properties (Fig. 1A). NAL (2.0, 0.2, and 0.02mg/kg, sc) administered with AMPH blocked the preference for the compartment paired with AMPH. The higher doses of NAL appeared to be aversive, in that rats avoided the compartment paired with this drug. The low dose, while having no aversive effects alone, still blocked place conditioning by AMPH (Fig. 1B). These data suggest that 1) AMPH is indeed reinforcing, 2) moderate doses of NAL are aversive, and 3) opiate receptor blockade can prevent the reinforcing properties of AMPH. Furthermore, these results suggest that endogenous opioids may be important in the reinforcing effects of AMPH, and support the possibility of interactions between endogenous opioids and catecholamines in reinforcement. (Supported by AFOSR grant 84-0325)




344.4 MORPHINE AFFECTS THE SUMMATION PROCESSES UNDERLYING THE PRODUCTION OF BRAIN STIMULATION REWARD. T.H.Hand and A.Laferrière*. Dept. of Psychology, McGill University, Montréal, Qué. Canada H3A 1B1

The effect of chronic morphine (5 mg/kg) on the sensitivity of the substrate for lateral hypothalamic self-stimulation (ICS) was determined by evaluating the effect of the drug on the current-frequency tradeoff function. The rationale for the use of this function is as follows: pulse frequency determines the firing rate of stimulated fibres passing through the effective area of stimulation, while current level determines the size of the stimulated area, and hence the number of fibres recruited. As one of these two variables is increased, the other can be decreased proportionally to maintain a predetermined level of ICS. If the rewarding efficacy of a train of pulses depends on the total number of firings of behaviourally relevant fibres, the reciprocal relation between current and frequency exists because as the firing rate of individual neurons is elevated, the number of neurons necessary to maintain ICS diminishes correspondingly. The tradeoff of current and frequency is thought to manipulate spatial and temporal summation processes (respectively) underlying ICS (Gallistel et al., Psychol. Rev. 88: 228, 1981).

Chronic morphine treatment is known to facilitate ICS, and although this facilitation is usually reported as increased ICS rate or decreased ICS threshold, neither of these drug-induced changes is very useful in determining what morphine actually does to the ICS substrate. On the other hand, morphine's effect (or lack thereof) on the current-frequency tradeoff function would be of considerable value in better understanding morphine-induced facilitation of ICS. Accordingly, the function was determined in 6 rats, and was redetermined after several days of chronic morphine treatment (1h post-injection). Each rat served as its own control. In all subjects, morphine reduced the current necessary to maintain half-maximal responding across a wide range of frequencies (28 - 200 Hz), with a maximal reduction at the lowest frequencies tested. This shift in the tradeoff function shows that morphine increases the sensitivity of the ICS substrate, especially when it is activated at low frequencies. It further shows that the drug enhances the spatiotemporal summation processes underlying ICS behaviour. The ability of morphine to increase ICS rate and decrease ICS threshold may be related to this enhancement.

We are currently evaluating the effect of morphine on the strength-duration curve and on other psychophysiological curves, and these results will also be presented.

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344.5 EFFECTS OF FOREBRAIN KNIFE CUT TRANSECTIONS ON MEDIAL FOREBRAIN BUNDLE SELF-STIMULATION IN THE RAT. Meg A. Waraczynski*(SPON: J.R. Stellar). Department of Psychology and Soc. Rel., Harvard University, Cambridge, MA 02138.

Data from psychophysical, autoradiographic, and electrophysiological experiments suggest that descending projections from the forebrain to the ventral tegmentum form an important part of the directly stimulated substrate for the rewarding effects of medial forebrain bundle (MFB) stimulation. Specifically, nuclei ventral and slightly caudal to the genu of the corpus callosum are promising candidates for the site(s) of origin of fibers carrying the reward signal (Shizgal, Bielajew, and Kiss, Neurosci. Abst., 6:422, 1980; Yadin, Guarini, and Gallistel, Brain Res., 266:39-50, 1983; Shizgal and Rompre, Neurosci. Abst., 10:310, 1984). This study investigates the effects of knife cut transections which disrupt efferents from these sites on MFB self-stimulation.

Rats are implanted with an MFB stimulating electrode, and an ipsilateral guide shaft cannula through which the knife cut is later made. Self-stimulation responding is evaluated using the reward summation function technique (Edmonds and Gallistel, JCPP,87: 876-883, 1974). The rat presses a lever for stimulation of various pulse frequencies. The resulting rate-frequency curve is then analyzed into two statistics: the maximum response rate (an index of performance capacity) and the frequency required to sustain half that rate. The latter statistic is called the "locus of rise" of the curve, and is interpreted as an index of the reward effectiveness of stimulation. Rats are tested until baseline responding stabilizes; they are then given the knife cut. Changes in maximum rate and locus of rise are observed on a daily basis for up to two weeks. Following testing, brains are sectioned in the sagittal plane, and the extent of knife cut damage is reconstructed on a single coronal section. Knife cut transections were chosen over electrolytic lesioning for this study, because this method allows one to produce selective damage in a small cross-sectional area while avoiding attendant rostrocaudal damage characteristic of lesions.

To date, 12 subjects have completed the experiment, with knife cuts at the level of the lateral preoptic area. Results from these rats show that lateral, ventral cuts in this area are more effective than medial cuts in attenuating MFB stimulation reward. The more medial, ineffective cuts completely transect the medial preoptic area and damage the medial portion of the lateral preoptic area, suggesting that these regions are probably not critical sources of descending fibers of the directly driven substrate. Implications of these results will be presented for the role of efferents from more anterior nuclei, which pass through the area of transection on their way to the medial forebrain bundle.

344.6 THE EFFECTS OF DIAZEPAM ON SELF-STIMULATION, ESCAPE FROM CONTINUOUS LATERAL HYPOTHALAMIC EXCITATION AND STIMULATION-BOUND FEEDING DISCRIMINATE BETWEEN PURE REWARD AND REWARD-ESCAPE RATS. S. E. Carden* and E. E. Coons (SPON: S. M. Feldman). Psychology Dept., New York Univ., New York, N.Y. 10003.

Pure reward and reward-escape rats, chronically implanted with lateral hypothalamic (LH) electrodes, were assessed for effects of Diazepam (DZ) on stimulation-bound feeding (SBF), barpressing rates for the onset of long and short trains of LH stimulation and for escape from continuous LH excitation. By testing across a range of behaviors, the question was raised whether pure reward and reward-escape rats display two discrete behavioral patterns or represent endpoints in a continuum of behavior which vary more in degree than in kind.

Sixteen combinations of currents and frequencies were used for all self-stimulation and escape conditions. Following the collection of baseline data and a period of drug acclimation, animals were retested with DZ (5 mg/kg, i.p.). Although pure reward rats barpressed at higher rates than reward-escape animals, the pattern of response magnitude showed the same configuration for both groups on baseline and drug trials, for long and short trains, whether plotted by current or frequency. After an initial sharp rise, responses plateaued and then decreased. In every condition, the combination of high current and high frequency reduced barpressing rates. These high stimulation levels either could be activating an aversive element within the LH reward system, or be providing positive reinforcement so powerful that it persists beyond the offset of stimulation. The similarity of response patterns in the two groups of animals would suggest that the neural substrate for reward is functioning in like manner for both groups and is comparably drug sensitive, but that there exists an additional aversive component in the reward-escape animals. This concept is supported by DZ trials demonstrating augmented reward for both groups but not displaying diminished escape in reward-escape rats.

The data in this study was confirmatory of previous research in this laboratory (Simson and Coons, Soc.Neurosci. Abstr., Vol. 9, Part 2, p.980, 1983) showing pure reward animals to be feeders and reward-escape animals to be non-feeders.

344.7 SELF-STIMULATION TRAIN DURATION RESPONSE FUNCTIONS MAINTAIN A STEP-LIKE APPEARANCE DESPITE 2.0 MSEC DURATION SPACING. R. A. Frank. Department of Psychology, University of Cincinnati, Cincinnati, OH 45221.

Self-stimulation train duration response functions typically show a dramatic, step-like increase from no responding to near maximal response rates as one increases train durations from sub-threshold to suprathreshold levels. This finding suggests that animals respond to the stimulation in an "all or none" fashion rather than titrating their response rates to the gradations in train duration. However, previous research has spaced the test durations by 10 msec, and it could be argued that if the spacing was reduced in the perithreshold region, a more gradual change in response rate would emerge. This hypothesis was tested by generating train duration response functions with test durations spaced at 2.0 msec intervals. Six male Sprague-Dawley rats implanted with bipolar stimulating electrodes in the ventral tegmental area (VTA) were trained to lever press for brain stimulation using a procedure that alternated 1.5 min test periods with 30 sec time-outs. Train durations ranging from 20 to 44 msec (in 2 msec increments) were presented in a descending order, along with a 0 msec (i.e., no stimulation) trial. Stimulation frequency and current intensity remained constant at 60 Hz and 50 $\mu$A, respectively. Two 28.0 min test sessions were run consecutively each day for three days. The data were analyzed by examining the response rates generated for each train duration during the final 30 sec of each trial. It was found that all six subjects produced the step-like functions that had been observed with the more widely spaced train durations. In fact, 93% of the trials produced response rates of either below 5 responses/30 sec or above 50 responses/30 sec. Additional work with these animals demonstrated that d-amphetamine shifted the train duration response functions to the left, but did not alter the shape of the curves. With amphetamine, 91% of the trials produced less than 5 or more than 50 responses/30 sec. The results clearly show that self-stimulating rats are extremely sensitive to variations in train duration. They also suggest that a critical train duration exists below which the rats are unwilling to respond and above which very vigorous responding is observed. Finally, it would appear that a 2.0 msec change in train duration is sufficient to cross over the critical duration value.

This research was supported by a University of Cincinnati URC grant to R. Frank.

344.8 INDIVIDUAL DIFFERENCES IN THE EFFECT OF MORPHINE ON TRAIN DURATION THRESHOLDS AND RESPONSE RATES IN SELF-STIMULATING RATS. A. Markou and R. A. Frank. Department of Psychology, University of Cincinnati, Cincinnati, OH 45221

In a previous study conducted in our laboratory, an effort was made to assess the effects of chronic morphine administration on train duration thresholds and response rates in self-stimulating rats. It was found that the magnitude and direction of morphine-induced changes in rates and thresholds varied substantially from animal to animal. This situation was further complicated by the failure of many animals to return to their pre-drug thresholds and response rates. The present experiment used higher doses of morphine (15 mg/kg) and a longer testing regimen in an attempt to clarify the results of the previous study. Eleven male Sprague Dawley rats implanted with bipolar stimulating electrodes in the ventral tegmental area or substantia nigra were trained to lever-press for brain stimulation using a procedure that alternated 1.5 min test with 0.5 min time-out periods. The brain stimulation train duration that was available during each testing period was varied from 20 to 140 msec in 10 msec increments. The train durations were presented in a random order during each testing session. Two 28 min testing sessions were run each day; the first 2 hr and the second 4 hr post-injection. During pre- and post-drug baseline periods normal saline was administered whereas 15 mg/kg morphine was injected (SC) on each of 15 consecutive drug testing days. Examination of the data, averaged across all animals, revealed that train duration thresholds were elevated during the initial days of morphine administration, the effect being more prominent during the 2.0 hr post-injection session. This depressive effect of morphine tended to tolerate over days. During the post-drug testing days, thresholds returned to the pre-baseline levels. Average response rates were markedly depressed during the first days of morphine administration. This depressive effect also tolerated over days and finally a facilitation in rate of responding became evident. Although the depressive effect was observed in both daily sessions, the facilitative effect was observed only during the second daily session. Response rates failed to return to pre-drug baseline levels despite extensive testing. Inspection of individual subject data revealed that the averaged data were not representative of the performance of all animals. The time course of facilitation and depression for both rates and thresholds was remarkably different from animal to animal. For example, several animals demonstrated decreases in thresholds after the administration of morphine while others exhibited marked threshold elevation during the same period. These results emphasize the importance of examing individual subject data when assessing the influence of morphine on self-stimulation behavior.

344.9 SELF-STIMULATION TRAIN DURATION THRESHOLDS PREDICT DEGREE OF SELF-DEPRIVATION. L. L. Wiggins & R. A. Frank. Department of Psychology, University of Cincinnati, Cincinnati, OH 45221.

It has often been assumed that self-stimulation thresholds provide an index of the attractiveness of brain stimulation. However, relatively few experiments have sought an explicit validation of this assumption. The present study assessed the relationship between self-stimulation train duration thresholds and the degree of self-deprivation observed during food/brain stimulation competition. Sixteen male Sprague-Dawley rats implanted with bipolar stimulating electrodes in the ventral tegmental area (VTA) were trained to lever press and nose poke for brain stimulation using a procedure that alternated 1.5 min stimulation periods with 30 sec time-outs. The animals were then reduced to 85% of their free-feeding body weights and tested with brain stimulation train durations that ranged from 30 to 150 msec in 10 msec increments. The order of train duration presentation was random, and an additional 0 msec (i.e. no stimulation) trial was included to measure free operant response rates. Each testing session lasted 28 min. Lever pressing and nose poking were used on alternate days. Once five sessions had been run with each operant, the rats were habituated to feeding in the testing chambers for three days. During this time, no brain stimulation was available. Subsequently, food and brain stimulation were available simultaneously during six food/brain stimulation competition sessions. Lever pressing and nose poking were alternated across test days and train durations were set at 250 msec for these sessions. It was found that train duration thresholds (defined as the train duration that supported 50% of maximal response rate) were significantly correlated with the degree of self-deprivation (defined as the mean amount of food consumed/session) for both lever pressing ($r = -.65$, $p < .01$) and nose poking ($r = -.80$, $p < .01$). These results support the claim that self-stimulation thresholds measure variations in the attractiveness of brain stimulation. They also suggest that the relationship between thresholds and self-deprivation does not depend on the operant that one chooses.

This research was supported by a University of Cincinnati URC grant to R. Frank.

344.10 SHORT- AND LONG-TERM SUMMATION CHARACTERISTICS OF ELECTRICAL SELF-STIMULATION REWARD. P.A. Mason and P.M. Milner (SPON: N.M. White). Dept. of Psychology, McGill University, Montreal, Quebec, H3A 1B1, Canada.

Using a Y-maze preference test paradigm, we examined the characteristics of the neural networks that integrate trains of rewarding stimulation pulses. Rats with mid-posterior lateral hypothalamic electrodes compared the rewarding effectiveness of various durations of a test reinforcement (0.15, 0.35, 0.55, 0.75, 1.05, 2.52, 4.52, 9.52, or 19.0 sec) to those of three durations of a standard reinforcement (0.50, 1.0, or 2.0 sec). At each duration of the test reinforcement its pulse frequency was adjusted so that the test reinforcement was chosen on approximately 50% of the trials when compared to the standard reinforcement. This procedure was repeated for each duration of the standard reinforcement. The standard reinforcement was delivered with a 100 Hz pulse frequency. The pulses were always 0.1 msec duration.

By changing the duration of the standard reinforcement while keeping the pulse frequency constant, we varied the stimulation magnitude to which the test reinforcement was being compared. This enabled us to examine the relationships between stimulation parameters both within a constant stimulation magnitude and across different stimulation magnitudes.

The data were inconsistent with a simple integrating system with a single decay time-constant, such as has sometimes been proposed (Gallistel, J. Comp. Physiol. Psychol., 92: 977-998, 1978). They can most parsimoniously be accounted for by two systems having quite widely differing time constants. The first integrator has a time-constant of approximately 450 msec and summates all activity produced by the stimulation. The second integrator has a time-constant of approximately 6.5 sec and preferentially summates stimulaton delived at low pulse frequencies. The reason this integrator responds poorly to high pulse frequencies may not have anything to do with the characteristics of the integrator itself, but rather to the long refractory periods of the fibers that provide input to this integrator. (Supported by NSERC Canda grant A66 to P.M. Milner).

344.11 COMPARISON OF NEUROBEHAVIORAL PARAMETERS OF BSR BETWEEN LATERAL HYPOTHALAMIC AND VENTRAL TEGMENTAL BRAIN SITES. M.J. Lewis and R.W. Phelps*, Dept. of Psychology, Howard Univerisity, Washington D.C. 20059, and Cortex Incorporated, Wellesley, MA., 02181.

Recent investigations of drug effects on brain stimulation reward (BSR) at lateral hypothalamic (LH) and ventral tegmental projection of the ventral noradrenergic bundle (VNB) brain sites have indicated differential effects at these sites. Data from the author's laboratory show that at VNB sites naloxone increases BSR threshold and decreases response rate; however, at LH sites the same doses have no effect on responding. Conversely, low doses of ethanol have no facilitatory effects on responding for VNB stimulation; however, such doses reduce BSR threshold for stimulation at LH sites. We report here a systematic investigation of BSR at both brain sites employing a sophisticated multifunctional brain stimulation system (Phelps and Lewis, Beh. Res. Meth. & Instr. 1982, 14(3), 323-328).

Male albino rats were implanted with platinum (PL) or stainless steel (SS) bi-polar electrodes using standard stereotaxic procedures. Electrodes were directed at lateral hypothalamic (LH) sites within the medial forebrain bundle or the ventral tegmentum (VT) posterior to the substantia nigra. Animals were shaped to lever-press for BSR at each site. Animals were trained initially under continuous reinforcement and then under a fixed ratio schedule of reinforcement.

BSR threshold rates were found to be lower and response rates higher for animals with LH electrodes in comparison to those with VNB electrodes irrespective of electrode type. Brain impedance measures were found to be lower at the LH sites with both PL and SS than at the VNB sites.

Electrode type seemed to affect stability in BSR. Animals that were implanted with PL electrodes and tested over a 9 month period showed unchanged BSR threshold. Animals with SS electrodes in the LH showed progressive increase in threshold over this period.

Brain impedance and BSR response rates showed no change with PL electrodes, whereas impedance increased and response rates became more variable over the 9 month period.

Examination of BSR threshold determination by descending current schedules and both ascending and descending schedules showed correlations of .92 for PL electrodes and .87 for SS electrodes between the two methods.

These data show site specific differences in BSR performance at LH and VNB brain sites which may explain previously reported differences in drug effects at these sites. Moreover, they indicate the importance of the type of electrode and the measurement of multiple parameters of BSR.

(Supported in part by DHHS grants RR07179 and DA 0216)

344.12 **PHARMACOLOGICAL EVIDENCE FOR CHOLINERGIC MODULATION OF THE SENSORY CUES ELICITED BY ELECTRICAL STIMULATION OF THE VTA.** **J.P. Druhan, M.T. Martin-Iverson, D. Wilkie*, H.C. Fibiger and A.G.Phillips.** Dept. Psychol.; Div. Neurol. Sci., Univ. of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

**Recent studies have shown that drugs which affect the functioning of dopamine neurons do not alter discriminated responding when discrete pulses of rewarding lateral hypothalamic (LH) or ventral tegmental area (VTA) brain stimulation are used as the discriminative stimuli. These results suggest that the discrete sensory events associated with delivery of brain stimulation to these areas are not related to the activation of dopamine neurons. The present study was designed to investigate whether cues produced by VTA stimulation involve a cholinergic substrate. Rats with VTA electrodes were trained to make a discriminated operant response (for a food pellet) on one of two levers following pulses of high intensity brain stimulation, or on the alternate lever after low intensity pulses. Each daily session included 90 trials, delivered at variable 20 sec intervals, with 4 pulses of either high or low intensity sine-wave stimulation being delivered randomly on different trials. Following training, the rats were given generalization tests in which intermediate current intensities wee administered on 20% of the trails. Stimulus generalization gradients were obtained for each animal after separate injections of physostigmine (.25 mg/kg and .5 mg/kg) and saline. Methyl-scopolamine (.5 mg/kg) was injected in conjunction with both doses of physostigmine to block the enhancement of peripheral cholinergic activity, thus each rat was also tested after administration of methyl-scopolamine plus saline to control for possible effects of this drug. The high dose of physostigmine (.5 mg/kg) produced a shift to the left in the generalization function as compared to that obtained under the saline condition. In the presence of the drug, lower intensity stimuli elicited responding on the lever appropriate for the high current intensity, indicating a possible augmentation of the stimulus property of a fixed intensity of brain stimulation. In contrast, neither the low dose of physostigmine nor methyl-scopolamine plus saline resulted in comparable changes in discriminated responding. Modulation of the discriminative stimulus properties of VTA stimulation by physostigmine may indicate a cholinergic involvement in the sensory properties of electrical stimulation in this region of the brain.**

**Supported by a grant (PG-23) from the Medical Research Council of Canada.**

344.13 **INCREASED DA RELEASE FOLLOWING FR10 ICSS WHEN COMPARED TO CRF ICSS AND CONTROLS.** **H. M. Fenton, D.B. Neill*, and J.B. Justice Jr. (SPON: C. K. Erickson) Department of Psychology, Emory University, Atlanta, GA 30322.**

**The dopamine (DA) theory of intracranial self-stimulation (ICSS) remains a parsimonious explanation for much experimental data. The theory states that each electrical stimulation activates a reward pathway, in which DA serves as a critical link. According to this theory, more stimulations should elicit greater DA release. However, analysis of tissue levels of the DA metabolite DOPAC after ICSS sessions have not supported this theory (Mitchell, et al., Life Sci. 30:1081-1085, 1982). Seiden and colleagues (Brain Res. 183: 403-419, 1980) have found that responding for water on partial reinforcement schedules (e.g. fixed ratio 5: FR5) increases DA release. ICSS studies predominately use continuous reinforcement schedules (CRF). Extending Seiden's results to ICSS, one could predict that responding on an FR10 schedule would elicit more DA release than responding on a CRF schedule. Our objective was to assess DA release in rats after ICSS responding on a CRF schedule or FR10 schedule vs. a home cage control condition. DA and DOPAC levels were measured regionally.**

**All rats were initially trained to bar press for lateral hypothalamic ICSS on a FR-10 schedule. Stimulation parameters were 0.5 msec biphasic square waves, 100 pps, 150 msec trains. Current intensities were adjusted to maintain low levels of responding (1500 per 22 min session), and this current was used throughout the experiment. When responding stabilized on the FR10 schedule, animals were put into one of three treatment conditions for 5 days: FR10-ICSS, CRF-ICSS or home cage controls. Thirty min after the session (to allow for DOPAC accumulation (Michael, Justice, and Neill, in press)) on the fifth day brains were rapidly removed, 1 mm slices were cut, and tissue punches taken ipsilateral and contralateral to the electrode. The following DA terminal regions were examined: olfactory tubercule, n. accumbens, anterior striatum, and central striatum. DA and DOPAC were measured using ion-pair reverse-phase HPLC-EC, and protein was assessed by the Lowry method.**

**T-test analysis of DOPAC/DA ratios per brain area revealed no differences when the two sides of the brain were compared (i.e., no assymetric release). No significant differences were found between home cage controls and CRF-ICSS for any brain site assayed. The FR10 animals had significantly higher DOPAC/DA ratios in the n. accumbens and anterior striatum, when compared to the CRF animals and the home cage controls. This was due to increased DOPAC levels. Our results do not support the DA theory of hypothalamic ICSS. However, these results are consistent with the idea of DA release during partial reinforcement.**

344.14 AVERSION-GATING SITES IN LATERAL HYPOTHALAMUS: AN ANATOMIC AND PHARMACOLOGIC STUDY. K.D. Carr and S. Uysal*. Dept. of Psychiatry, NYU Med. Ctr., New York, N.Y. 10016.

Lateral hypothalamic (LH) electrical stimulation may diminish the aversive dimension of pain through operation of a supraspinal mechanism ('aversion-gating'). For example, LH stimulation inhibits a post-stimulus vocalization response (PSV) elicited by brief tail shock (Carr and Uysal, 1985). PSV is organized in forebrain structures and is considered an "affective" response to pain. Simpler vocalization and motor responses, organized in hindbrain and spinal cord respectively, are unaffected. LH stimulation also diminishes aversion that is induced supraspinally by stimulation in pain-related nucleus gigantocellularis (NGC) (Carr and Coons, 1982). In the present study, the relationship between electrode placement and potency of the aversion-gating effect was evaluated. In the tail-shock paradigm (N=19), LH stimulation intensities were at threshold for ICSS and therefore equated across rats with regard to positive reinforcement. However, the % elevation in PSV threshold varied greatly among rats (25-200%). Electrodes associated with the greatest effects were localized to the dorsolateral quadrant of the MFB. In the NGC paradigm (N=19), LH electrodes in which low stimulation intensities reduced NGC-escape rate by a criterion 50% were also localized to the dorsolateral MFB. Sites in which ICSS could be elicited at low intensities were localized to a central region that extended across the width of the MFB. The dorsolateral region associated with strong aversion-gating effects is distinguished by proximity to dopamine-containing (Ungerstedt, 1971) and dynorphin-containing (Weber et al., 1982) neurons. In the NGC paradigm, pimozide, the dopamine antagonist, had no effect on aversion-gating at 0.125 mg/kg (i.p.) but reduced the effect by about 10% at 0.25 mg/kg (N=4). Both doses are reported to effect substantial decreases in LH ICSS (Fouriezos et al., 1978). In the tail-shock paradigm, the opioid antagonist naltrexone (10 mg/kg, s.c.) substantially reduced (50-100%) the effect of LH stimulation (N=6). Naltrexone did not alter thresholds for ICSS in these LH electrodes. In the NGC paradigm, naltrexone produced a small decrease in aversion-gating in 3 rats (15-20%) and a larger decrease in 3 others (40-80%). Electrodes for the latter 3 were localized to the dorsolateral MFB while the others were scattered and distant from the dorsolateral locus.

These results suggest that 1) the population of LH neurons which mediates inhibition of NGC-induced aversion may be the same as that which mediates inhibition of PSV, 2) the population which mediates aversion-gating effects is not identical to the population which mediates ICSS, 3) unlike ICSS, dopaminergic activity may not play a key role in aversion-gating, and 4) unlike ICSS, opioid activity is involved in the aversion-gating effect and is preferentially stimulated by electrodes in the dorsolateral MFB focus of the aversion-gating system.

344.15 REFRACTORY PERIODS AND CONNECTIVITY OF THE SUBSTRATE FOR BRAIN STIMULATION REWARD AND STIMULATION INDUCED FEEDING. A. Gratton* and R.A. Wise (SPON: P. Shizgal). Center for Studies in Behavioral Neurobiology, Concordia University, Montreal, Canada H3G 1M8.

The refractory periods (RPs) of the directly activated medial forebrain bundle (MFB) substrates for brain stimulation reward (BSR) and stimulation induced feeding (SIF) were compared at the same site of stimulation. The RPs were inferred behaviorally using a paired-pulse technique, where the animal's response to a train of pulse pairs was compared to its response to a train of single pulses. Systematically varying the delay (C-T interval) between the two constitutent pulses (C and T pulses) of the paired-pulse condition yielded a distribution of RPs of the BSR and SIF-relevant fibers. The data show that BSR and SIF are subserved by fibers having essentially the same distribution of RPs. Furthermore, as was the case for BSR, a flat portion in the SIF RP curve was found between 0.6 and 0.7 msec. Similar RP estimates for SIF and BSR were obtained at a variety of MFB sites which extended from the ventral tegmental area (VTA) to the anterior lateral hypothalamus (LH). This last finding is consistent with the idea that SIF, like BSR, is subserved by MFB fibers extending between LH and VTA.

To determine if SIF in LH and VTA is subserved by a common set of fibers, a variation of the paired-pulse technique was used in rats feeding in response to unilateral stimulation of both VTA and LH. In this preparation a train of C-pulses was applied to one electrode while a train of T-pulses was applied to the other electrode at a variety of C-T intervals. Connectivity between the two stimulation sites was inferred from an abrupt rise in T-pulse effectiveness which occurred at C-T intervals longer than 1.5 msec. This C-T interval is assumed to reflect the sum of the refractory period and the collision time between the two electrodes. Estimates of refractory periods (0.4 to 2.0 msec) and conduction velocity (approx. 2.45 m/sec) were consistent with those associated with BSR. Our data suggest that SIF-relevant fibers do extend without synaptic interruption between LH and VTA. The fact that T-pulse effectiveness increased at the same critical C-T interval when the T-pulse was applied to either the LH or VTA electrode strengthens this conclusion.

344.16 BOUNDARIES AND HOMOGENEITY OF BRAIN STIMULATION REWARD SITES IN THE ANTERIOR MEDIAL FOREBRAIN BUNDLE. Adina Blander* and Roy A. Wise. (SPON: Robert B. Malmo) Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, Montreal, Quebec.

Recent studies have suggested that the directly activated mechanism of medial forebrain bundle brain stimulation reward involves fibers projecting in a rostro-caudal direction with the cell bodies located somewhere anterior to the lateral hypothalamus. The present study was a detailed mapping of brain stimulation reward sites in the anterior hypothalamic medial forebrain bundle. Forty adult male Long-Evans rats were tested using moveable electrodes that allowed testing at .25mm intervals over a 2.5mm dorsal-ventral range. Reward thresholds, response rates at various stimulation intensities, and stimulation side effects (including locomotion and seizures) were noted for each of the 350 sites tested. Positive sites were found at most levels of the MFB from the lateral hypothalamus to the preoptic-area. Many sites in the lateral pre-optic area were negative. Within the range of anterior-posterior coordinates tested, sites at .05mm from the midline were negative. Positive sites ranged from 1.0mm to as far as 3.0mm lateral. Dorsal-ventral boundaries varied substantially with medial-lateral and anterior-posterior coordinates. No evidence was found to support the view that the reward fibers arise from a single, anatomically restricted group of cell bodies at the head of the medial forebrain bundle.

344.PO SEROTONERGIC CONTROL OF AGGRESSIVE "STATE" BY THE ACCUMBENS/PREOPTIC AREA (APOA) 3. STATE-ASSOCIATED CHANGES IN $5HT_2$ RECEPTORS M. Potegal and H.R. Wagner. N.Y. State Psychiatric Institute and the Columbia University College of Physicians and Surgeons, N.Y., N.Y.

"Priming" a female hamster by allowing it one biting attack on a drug-treated target hamster reduces the latency of the subsequent attack. "Satiating" a hamster with a series of targets until it meets a criterion of 3 successive target presentations without attack increases the latency and decreases the number of subsequent attacks. The half life of the priming effect is approximately 12 hr; that of the satiation effect is 24 hr (Potegal and Popken, Behav. Processes, In press).

We have previously found bidirectional priming/satiation-related changes in APOA 5HT uptake that were neuroanatomically, neurochemically, and behaviorally specific (Barkai, et al, Neurosci Abst., 10: 1170, 1984). We now report similar priming/satiation-associated changes in $5HT_2$ receptors as indicated by ketanserin binding.

Ketanserin binding was characterized in pooled hamster APOA. Following homogenization and centrifugation (40,000g x 10 min), membranes were suspended in 250 volumes (W/V) of iced 50 mM Tris-HCl (pH 7.6 at 25°C). Twelve $^3$H-ketanserin concentrations between 0.1-8.0 nM were added to aliquots containing approximately 200 µg protein, with or without $10^{-5}$M methysergide, to a final assay volume of 1 ml. Samples were incubated to equilibrium (30 min at 23°C) in a shaker. The assay was terminated by vacuum filtration. The Whatman (GF/B) filters were washed, dried, suspended in Aquasol (NEN) and counted by liquid scintillation spectrometry. Scatchard analysis of specific binding, defined as the difference in total binding in the presence and absence of methysergide, yielded a $B_{max}$= 48.2 fmol/mg protein and a $K_d$=1.11 nM.

Eighteen hamsters were subsequently matched into triplets by aggression screening score. Members of each triplet were sacrificed at the same point in the light cycle after being either attack-primed, attack-satiated or given a control manipulation. There was an 8% increase in specific APOA binding of 0.5 nM $^3$H-ketanserin in primed animals (21.6 fmol/mg protein) and an 18% decrease in satiated animals (16.3 fmol/mg protein) relative to control levels. The difference between matched pairs of the primed and satiated animals was significant [$t(5)=3.3$, $p<.05$]. There were no significant differences in APOA $^3$H-5HT binding.

It is noteworthy that for both ketanserin binding and 5HT uptake, the difference between the primed and satiated groups was approximately 20% and the change from control levels in the satiated group was slightly more than twice that in the primed group. The larger satiation-associated changes are consistent with the longer half life of the satiation effect. The regulation of aggressive state apparently involves a complex series of changes in serotonergic function.

345.1 DESCENDING PAIN INHIBITION: OPIATE RECEPTOR SUB-TYPES IN THE PAG. D.J.Smith, J.M. Perrotti*, A.L. Mansell*, J.M. Scalzitti* and J.T. Long*. Dept. of Anesth., WVU Med. Cent., Morgantown, WV 26506.

An important neuronal pathway involved in the analgesic response to morphine-like narcotics (mu, μ, opiate receptor agonists) is the pain inhibitory system originating in the periaqueductal gray (PAG) region of the midbrain and descending into the spinal cord dorsal horn (Yaksh & Rudy, Pain 4: 299, 1978). However, little is known of the contribution of the PAG in the antinociceptive action of narcotics with preferences for other opiate receptors. Thus, prototypic agents for these subtypes were examined for their ability to activate this spinopetal system. These included: 1) d-alanine, d-leucine enkephalin (DADL), Delta (δ) receptor: 2) ethylketocyclazocine (EKC), Kappa (K) receptor; 3) n-allyl normetazocine (NAM), Sigma (σ) receptor and 4) ketamine, an anesthetic with opiate receptor properties similar to those of the benzomorphan (i.e., K and σ) narcotics (Smith, et al.: Neuropharmacol. 21:604, 1982, Pain 21: 353, 1985). The drugs were either 1) microinjected directly into the PAG and their analgesic effect evaluated using the tail-flick reflex or 2) were given systemically and the sensitivity of their action to the microinjection of naloxone was determined.

The microinjection of morphine caused a dose-dependent increase in tail-flick latency (TFL was twice the pre-drug value with 3 nmoles and reached "cut-off", 15 sec, with 24 nmoles). Naloxone administered either systemically or by sequential microinjection (1 hour after the agonist) antagonized morphine's action. In contrast to morphine, EKC (10-100 nmoles), NAM (4-40 nmoles) and ketamine (0.4 - 420 nmoles) failed to produce analgesia. However, DADL was slightly effective at the highest dose (40 nmoles) tested. This suggested that its action may be related to higher and less specific doses interacting with μ receptors through which morphine acts.

Systemic administration of the agonists, with naloxone being given by microinjection, confirmed results obtained with some of the drugs. However, analgesia from systemic EKC (10 mg/kg,s.c.) was transiently attenuated by the antagonist, suggesting that an activation of intrinsic (PAG) opiate nerves may have occurred via an action of the agonist on convergent neuronal processes.

In conclusion, only drugs that interact functionally as μ (morphine-like) or partial μ agonists (e.g., potentially DADL in high doses) will be effective in inducing activation of the descending inhibitory nerves originating in the PAG.

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345.2 MONOAMINE AND OPIATE INVOLVEMENT IN MIDBRAIN INHIBITION OF SPINAL NOCICEPTIVE NEURONS IN THE RAT. E. S. Culhane*, R. Banisadr* and E. Carstens (SPON: J. D. Miller). Dept. Animal Physiology, Univ. Calif., Davis, CA 95616.

Electrical stimulation of the midbrain periaqueductal gray (PAG) and more lateral reticular formation (LRF) inhibits spinal dorsal horn neuronal discharges to noxious skin heating and produces analgesia. To determine if inhibition from these two areas is pharmacologically distinct, we tested whether antagonists of three putative neurotransmitters-endogenous opiates, serotonin, and noradrenaline-block or reduce inhibition from PAG or LRF.

Responses of 38 single dorsal horn units to noxious skin heating (50-54°C, 10 s) of the hind footpad were recorded in the lumbosacral enlargement of rats continuously infused with sodium pentobarbital. Responses with or without PAG or LRF stimulation (100 msec trains at 100 Hz, 3/s, 25-400 μA) were recorded alternately at 2 min intervals. Inhibition was expressed as the ratio of the unit's response to heat during midbrain stimulation/unit's response to heat in the absence of stimulation. Once a stable degree of inhibition was obtained from PAG and/or LRF, either the opiate antagonist naloxone (5-10 mg/kg), the serotonin antagonist methysergide (2-6 mg/kg) or the adrenergic antagonist phentolamine (2-4 mg/kg) was injected via the femoral vein. Inhibition was said to be reduced post-drug (+) if it was > 20% below the pre-drug level of inhibition; a change of < 20% was called no effect (o).

Results for each unit tested to date are tabulated below. Methysergide reduced PAG inhibition in 2 additional units in which LRF was not tested, and phentolamine reduced LRF inhibition in 2 units in which PAG was not tested.

| DRUG | PAG↓/LRFO | PAG↓/LRF↓ | PAGO/LRF↓ | PAGO/LRFO |
|---|---|---|---|---|
| naloxone: | 1 | 3 | 1 | 7 |
| methysergide: | 5 | 1 | 0 | 8 |
| phentolamine: | 0 | 1 | 2 | 4 |

Reduction in inhibition by naloxone was apparently independent of the stimulation site within PAG or LRF. The inconsistent effects of naloxone indicate that endogenous opiates are not primarily involved in mediating inhibition from these areas.

The ability of methysergide to frequently reduce inhibition from PAG, but not LRF, suggests a role for serotonin in mediating inhibition from the PAG, although methysergide's lack of effect in other units indicates that other transmitters may also be involved. Likewise, while the data with phentolamine suggest a partial adrenergic involvement in inhibition from LRF, noradrenaline is certainly not the sole mediator. We are currently studying possible cholinergic involvement in descending inhibition, as well as a possible spinal site of action of monoamine antagonists (via intrathecal administration) to affect midbrain inhibition.

Supported by NIH grants NS 20037 and NS 19330.

345.3 CHOLINERGIC AND TRH INTERACTIONS UPON OPIATE AND NONOPIATE ANALGESIC MECHANISMS. R.J. Bodnar, P.D. Butler, E.S. Sperber, E. Kramer, P.E. Mann, M.T. Romero* and L.S. Truesdell. Dept. of Psychology, Queens College, CUNY, Flushing, NY 11367.

Both acetylcholine and thyrotropin-releasing hormone (TRH) modulate various forms of analgesic responses. For instance, while cholinergic muscarinic receptor blockade with scopolamine reduces the analgesic responses following either inescapable foot shock or cold-water swims (CWS), intracerebroventricular (ICV) TRH potentiates these responses. The present experiments extend these findings, and also demonstrate that cholinergic and TRH analgesic mechanisms interact. The first experiment compared the abilities of non-analgesic doses (50 ug, ICV) of TRH, its analog (RX 77368) and its metabolite (diketopiperazine: DKP) to potentiate CWS (21 C for 3.5 min) analgesia on the tail-flick and jump tests. TRH and DKP significantly potentiated CWS analgesia on both tests 30 min following the swim, while the longer-lasting analog (RX 77368) potentiated CWS analgesia across the entire 2 h time course. These effects could not be attributed to hypothermic changes. The second experiment examined the effects of a non-analgesic TRH dose (50 ug, ICV) upon pilocarpine (10 mg/kg, IP) analgesia on the tail-flick and jump tests. TRH significantly potentiated analgesia on the tail-flick, but not the jump test 30 and 60 min after injection of the muscarinic receptor agonist. Finally, given the inconsistent reports of mucarinic receptor involvement in opiate analgesia, the third experiment examined whether scopolamine (10 mg/kg, IP) pretreatment altered analgesia induced by the delta receptor agonist, d-ala,d-leu-enkephalin (DADL: 40 ug, ICV), the epsilon receptor agonist, beta-endorphin (BEND: 1 ug, ICV) and the mu receptor agonist, morphine (MOR: 5 mg/kg, SC). While scopolamine failed to alter BEND analgesia on the jump test, it significantly potentiated both DADL and MOR analgesia. Therefore, while acetylcholine and TRH each modulate both opiate and nonopiate forms of analgesia, they also interact with each other in these processes. (Supported by PSC/CUNY Grant 6-64187.)

345.4 RESPONSES OF SPINOTHALAMIC TRACT CELLS TO OPIATES AFFECTING DIFFERENT RECEPTOR SUBCLASSES. W.S. Willcockson*, J. Kim*, H.K. Shin*, J.M. Chung and W.D. Willis. Marine Biomedical Institute, Univ. Texas Medical Branch, Galveston, TX 77550.

Characterization of opiate actions on nociceptive cells of the spinal cord is important for identifying analgesic mechanisms and more selective drugs for pain relief. Opiates can produce analgesia at the spinal cord level (review Yaksh 1981) and have also been shown generally to inhibit nociceptive neurons in the dorsal horn when given systemically or applied iontophoretically (reviews North 1979, Duggan and North, 1984, Zieglgansberger 1984). Evidence now exists for multiple opiate receptors (reviews Kosterlitz 1983, Martin 1984). The relationship of these different receptors to nociceptive mechanisms is unclear although it is believed that opiates of the mu (μ) and delta (δ) subtypes cause analgesia. In the present study, we have iontophoretically applied representative agonists of 4 different subclasses onto cells of the spinothalamic tract (STT). Morphine (MOR) was the prototypic μ agonist. Dynorphin 1-13 fragment (DYN) was used as a kappa (k) drug (Chavkin et al. 1982). [D-Ala$^2$] met-enkephalinamide (MKN) was the δ agonist. The sigma (σ) agonist N-allylnormetazocine (NAM) was also compared, as was phencyclidine (PCP) which binds σ sites and has actions similar to NAM on dorsal horn interneurons (Zukin and Zukin 1979, Quirion et al. 1981, Lodge and Anis 1982).

Experiments were performed in anesthetized monkeys (Macaca fascicularis). The opiates were usually tested against activity evoked by pulsed release of GLU although effects on responses evoked by noxious mechanical or electrical stimuli were included.

While MOR was found to have predominantly inhibitory actions on STT cells, excitatory and mixed inhibitory and excitatory effects were seen. More intriguing was the ability of MOR to cause multiple single or biphasic responses on single STT cells, depending upon either current strength or change in position of the electrode relative to the cell body. DYN also produced inhibitory or excitant actions on the GLU activity of STT cells including multiple actions on single cells depending on dose or position. MKN, PCP and NAM, on the other hand, inhibited all cells studied, although some biphasic actions were also noted. Iontophoretic naloxone (NLX) at times antagonized the MOR effects but had little effect on the action of the other drugs.

The multiple effects of the opiates on STT neurons may be due to actions at multiple receptors. The selectivity, however, of most of these agents for their corresponding receptors is incomplete. Future assessment of opiate action on nociceptive cells will be aided by studies with more selective receptor agents.

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345.5 DISCOVERY OF A NON-OPIOID ANALGESIC WITH ANTIDEPRESSANT PROPERTIES, TAZADOLENE SUCCINATE. J.Szmuszkovicz*, P.F.VonVoigtlander*, R.A.Lewis*, M.C.Ochoa*, and H.J.Triezenberg* (SPON: E.D.Hall). CNS Research, The Upjohn Company, Kalamazoo, MI 49001.

Tazadolene succinate, 1-(2-(phenylmethylene)cyclohexyl)azetidine butanedioate, was synthesized as part of a program aimed at the discovery of non-opioid, centrally acting analgesics. Preliminary evaluation in mice revealed that tazadolene (T) has analgesic (HCl-induced writhing antagonism), but also antidepressant-like (yohimbine and apomorphine potentiation and oxotremorine antagonism) activity. Further evaluation in rats and mice confirmed and extended these observations. For example, in the hot plate test, T was effective against both a low (49.5°C) and a high (54.5°C) intensity stimulus. Subcutaneously, potency of T on these tests exceeds that of pentazocine (P) and approaches that of morphine (M); orally T was more potent than either P or M. This activity was confirmed in a writhing assay in rats, but T was inactive, or only weakly active, in analgesic tests (mouse tail flick and rat tail immersion) involving a spinal reflex response. In the hot plate and writhing assays, the antinociceptive activity of T was not altered by pretreatment with opioid antagonists. Repeated dosing with T did not lead to a diminished analgesic response or to hyperalgesia upon cessation of treatment. This is in contrast to M and P. Moreover, animals tolerant of M displayed no cross-tolerance to T, and T decreased withdrawal hyperexcitability in mice made physically dependent on M. These properties suggest that T may be particularly useful in the treatment of chronic pain. Chronic pain is often associated with depression, thus the antidepressant properties of T may be of added importance. T is more than twice as potent as imipramine in a behavioral despair test, and is an effective blocker of the uptake systems for serotonin, dopamine, and norepinephrine both in vitro and in vivo. Thus T is an analgesic as well as an antidepressant-like agent in animals, and may have special utility in the treatment of chronic pain in man.

345.6 CENTRAL ANTINOCICEPTIVE EFFECT OF ACETYLSALICYLIC ACID AND PARACETAMOL. K. Hole*, S. Hunskaar* and O.B. Fasmer* (SPON: A. Njå). Dept. Physiol., Univ. i Bergen, N-5000 Bergen, Norway.

The analgesic effects of non-steroid anti-inflammatory agents are thought to be peripheral, however, experiments using central application of aspirin-like drugs indicate that a central action may also exist.

Intrathecally administered substance P (2.5 ng in 5 µl), or capsaicin (10 ng), a compound that releases substance P from primary sensory afferent terminals, elicited a pain-related behavioral response consisting of vigorous biting, licking and scratching of the caudal part of the body in mice (Fasmer, O.B. et al., Neuropharmacol., 22:485, 1983). This response is assumed to be nociceptive and similar to behavior induced by a clearly noxious stimulus (Hylden, J.L.K. and Wilcox, G.L., Brain Res., 217:212, 1981). Pretreatment of the mice with intraperitoneally injected acetylsalicylic acid (300 and 400 mg/kg), paracetamol (300 and 400 mg/kg) and morphine (2.5 and 5 mg/kg) reduced the responses in a dose dependent manner. The highest doses of the drugs reduced the number of bites, licks and scratches to approximately one third of the control values elicited by substance P, and to approximately one half when this behavior was elicited by capsaicin.

These results show that acetylsalicylic acid and paracetamol as well as morphine may act in the spinal cord or supraspinally to inhibit behavioral responses that probably are pain-related, suggesting a central site of action for at least part of the antinociceptive effects of all these drugs. This analgesia is probably mediated by a postsynaptic substance P sensitive mechanism both for the mild analgesics and for morphine.

345.7 ACTIVITY OF NEURONS IN THE PERIAQUEDUCTAL GRAY DURING WITHDRAWAL FROM NOXIOUS HEAT. M.M. Heinricher, Z.-F. Cheng* and H.L. Fields. Depts. of Physiology and Neurology, Univ. of California, San Francisco, CA 94143.

The midbrain periaqueductal gray (PAG) and the rostral ventromedial medulla (RVM) are important links in a neuronal network which modulates nociceptive transmission. In the RVM, two classes of neurons have been identified which show changes in activity at the time of the tail flick response (TF) elicited by noxious heat (Fields, et al., J. Neurosci., 1983, 3, 2545). We now report that a significant number of neurons in the PAG region also show changes in activity which are related to the TF.

Male Sprague-Dawley rats (275-325g) were anesthetized with pentobarbital (60 mg/kg, i.p.) and a microelectrode was stereotactically placed at the dorsal border of the PAG. Following surgery, the animals were maintained in a lightly-anesthetized state by continuous infusion of methohexital at a rate (15-30 mg/kg/hr, i.v.) which prevented signs of discomfort and allowed a stable TF latency of 4-5 sec. The electrode was then lowered until TF suppression (10 sec cut-off) was obtained at stimulation currents of 10 µA or less (400 µsec pulses, 50 Hz continuous trains). Midbrain units were isolated and responses to noxious and non-noxious cutaneous stimulation determined. The units were then characterized according to the classification system used in the RVM. Cell activity, time of TF occurrence and tail temperature were stored during 5 repetitions of the heat stimulus. Peri-response and peri-stimulus histograms were plotted with respect to the TF and tail temperature, respectively.

A significant number of neurons in the PAG region showed changes in activity related to the TF. Two classes of TF-related neurons, similar to those described in the RVM, were identified in the PAG region. "On-cells," which displayed an abrupt increase in firing just prior to the TF, comprised approximately 20% of the cells recorded to date. "Off-cells," which showed an abrupt pause just prior to the TF, made up approximately 3% of the cells recorded. The remaining PAG neurons usually had no cutaneous receptive field and showed no change in discharge correlated with the TF.

Thus, cells with changes in activity related to the TF are present in the PAG region as well as in the RVM. The PAG has a large projection to the RVM, and microinjection of morphine in the PAG increases activity of RVM off-cells and decreases that of RVM on-cells. Thus, it is likely that on- and off-cells in the PAG participate in the well-documented modulation of nociceptive reflexes by the PAG and that this action is mediated by TF-related neurons in the RVM.

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345.8 THE EFFECT OF STIMULUS PARAMETERS AND ATTENTION ON THE MONKEY'S ABILITY TO DETECT SMALL INCREASES IN TEMPERATURE IN THE NOXIOUS RANGE. D.R. Kenshalo, Jr., R. Bates* and R. Dubner. Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20205.

In monkeys, detection latencies to small incremental temperature shifts in the noxious range provide an indirect measure of the perceived intensity of sensation (Bushnell et al., J. Neurosci., 1985, in press). The present study systematically examined the relationship between the intensity of noxious thermal stimulation and the monkey's ability to detect subsequent small temperature changes.

Four monkeys were trained in a psychophysical task to detect small temperature changes in the noxious range. A trial was initiated when the monkey pressed an illuminated panel button. Subsequently, a contact thermode, positioned on either the upper lip or on the volar surface of the wrist, increased in temperature from a baseline of 38°C to temperatures of 44°, 45°, 46° or 47°C (T1). After a variable time period of 3 to 9 sec, the thermode increased an additional 0.1°, 0.2°, 0.4°, or 0.6°C (T2). The monkey received 0.3 cc of fruit juice for releasing the button within 2.4 sec of the onset of T2. The detection latency was defined as the amount of time elapsing between the onset of T2 and the release of the button.

For all monkeys, as the first temperature shift (T1) increased, the T2 detection threshold systematically decreased from 0.6°C at a T1 of 44° to approximately 0.1°C at a T1 of 47°C. The median detection latencies were also inversely related to the intensity of T2 for suprathreshold temperature shifts. In addition, the detection latency following the onset of T2 was dependent on the T1 level. The results were similar for the face and the arm.

The influence of attentional factors on the detection of noxious thermal stimuli was studied by signaling the detection difficulty of the T2 stimulus. Near threshold stimuli (T2 = 0.2°-0.4°C) were signaled by a red light, while suprathreshold stimuli (T2 = 0.8°-1.2°C) were signaled by a blue light. Signaling the monkey that a difficult detection was about to occur resulted in a consistent decrease in the median detection latency and increase in the probability of detection for near threshold T2 stimuli. The median detection latency did not change for suprathreshold T2 stimuli.

These data suggest that monkeys are exquisitely sensitive to small temperature changes in the noxious range and that sensitivity improves in the noxious range of 45° to 47°C. Furthermore, detection latencies, which provide a measure of the perceived intensity of noxious stimuli, are systematically altered by attentional factors.

345.9 EFFECTS ON NOCICEPTIVE THRESHOLD OF THIP AND BICUCULLINE MICROINJECTED IN THE NUCLEUS RAPHE MAGNUS. E.J. Drower and D.L. Hammond. Dept. of Biological Research, G.D. Searle & Co., Skokie, IL 60077.

4,5,6,7-tetrahydroisoxazolo-[5,4-c]-pyridin-3-ol (THIP) is a GABA agonist reported to produce analgesia in the rodent and primate. A previous study from this laboratory determined that THIP did not act at the spinal level to produce analgesia. However, a supraspinal site of action was not excluded. The present study sought to determine whether the analgesic action of THIP was mediated supraspinally by neurons of the nucleus raphe magnus (NRM) and nucleus reticularis paragigantocellularis (NRPG), two medullary nuclei implicated in the regulation of nociceptive threshold.

Male Sprague-Dawley rats were implanted with a microinjection guide sheath aimed at the NRM or NRPG. One week later, either saline, the GABA agonist THIP (0.3-1.0 µg), or the GABA antagonist bicuculline (0.1-1.0 µg) was injected in a volume of 0.5 µl at sites in the NRM or NRPG. Alterations in nociceptive threshold were determined by measurements of tail flick latency (TFL), hot plate latency (HPL) and responsiveness to noxious pinch prior to and at fixed intervals after microinjection. Microinjection of saline did not significantly alter TFL, HPL or responsiveness to pinch. Microinjection of THIP in the NRM and NRPG decreased TFL and increased responsiveness to noxious pinch, but did not significantly alter HPL. In contrast, microinjection of bicuculline in the NRM and NRPG increased TFL and reduced responsiveness to noxious pinch; HPL was unaffected. These data suggest that neurons of the NRM and NRPG that regulate nociceptive threshold are subject to a tonic, GABA-ergic input. Removal of this input, by microinjection of bicuculline, produced analgesia, suggesting that the GABA-ergic input to the NRM and NPRG is inhibitory. These data further indicate that the analgesic activity of THIP is not mediated by neurons of the NRM and NRPG as microinjection of THIP at these sites produced hyperalgesia, rather than analgesia. Supported by G.D. Searle & Co.

345.10 ENDOGENOUS ANALGESIA IN THE PREGNANT RAT: AN ARTIFACT OF WEIGHT-DEPENDENT MEASURES? J.L. Dahl, B.W. Silva*, T.B. Baker*†, and S.T. Tiffany*†. Departments of Pharmacology and Psychology†, University of Wisconsin-Madison, Madison, WI 53706.

It has been reported that pain thresholds increase during pregnancy and parturition in the rat and that these increases can be prevented by administration of the opioid antagonist, naltrexone [Science 210, 193 (1980)]. These observations merit careful examination because they suggest that pregnancy activates a unique form of endogenous analgesia: one which is tonic, opioid-mediated, yet not associated with the development of tolerance. We used five different measures of nociceptive response across multiple experiments to compare the responsiveness of pregnant and nonpregnant female rats to painful stimuli. Pregnant and nonpregnant animals did not differ in response when assessed by tail-flick, tail-shock vocalization, and hot-plate tests. When nociception was assessed with a computerized flinch/jump procedure or with a jump-threshold test, there were significant differences between the responses of pregnant and nonpregnant control animals. However, a close inspection of the data showed that body weight differences could account for the apparent differences in the pain responsivity of pregnant and nonpregnant rats observed with these measures. Body weight accounted for a greater portion of variance in jump-response data than did pregnancy status. Furthermore, neither acute naloxone or chronic naltrexone administration blocked the increase in jump thresholds observed in pregnant rats, nor would we expect them to if the jump-threshold changes are determined wholly or partially by weight changes. In summary, we can find no evidence of an endogenous analgesia of pregnancy; instead, our results suggest that findings of a diminished response to painful stimuli in pregnant rats may be an artifact related to the greater body mass/weight of pregnant animals. Supported by funds provided by the Research Committee of the University of Wisconsin Graduate School to JLD.

345.11 EX VIVO INHIBITION OF MOUSE BRAIN PROSTAGLANDIN E2 FORMATION BY NON-OPIOID ANALGESICS. Richard A. Ferrari, Concetta Zobre*, Mary J. Connell* and Susan J. Ward*. Department of Pharmacology, Sterling-Winthrop Research Institute, Rensselaer, New York 12144

Prostaglandin E2 (PGE2) is known to be hyperalgesic. Reduction in PGE2 levels locally at the site of injury and/or in the brain is thought to be the mechanism of action of non-steroidal analgesics. A new animal model was developed to assay the effect of selected analgesics on post mortem PGE2 increases in mouse brain in a test system which closely approximates in vivo conditions.

The ex vivo synthesis of PGE2 from endogenous arachidonic acid in post mortem mouse brain was determined after incubating the brain at room temperature. The reaction was stopped by freezing on Dry Ice or by immersion in liquid nitrogen. Brains were homogenized in the frozen state in pH 3.5 citrate containing EDTA and indomethacin. Prostaglandins were adsorbed onto and then eluted from C18 Bond Elut columns (recoveries were 93 to 100%) prior to radioimmunoassay (sensitivity = 0.25 pg/assay tube).

The ex vivo PGE2 synthesis increased with time and plateaued at 2 min post mortem during which time zero control levels of PGE2 rose 20-fold from an average of 0.16 to 3.3 pg/mg brain. The ability of selected analgesics to prevent this post mortem formation of PGE2 after oral drug intubation was tested. Time course experiments demonstrated that a post drug treatment time of 30 min was optimal. The $ED_{50}$ values, in mg/kg as free acids, were: indomethacin 0.13, zomepirac 0.32, naproxen 1.7, ibuprofen 2.4, aspirin 35 and acetaminophen 113.

Minimal changes in time zero PGE2 levels were found at 30 min post-treatment p.o. with aspirin (1 to 300 mg/kg), indomethacin (0.1 to 3 mg/kg),. zomepirac (3 to 300 mg/kg) or acetaminophen (30 to 1000 mg/kg) when compared to vehicle treated controls.

The $ED_{50}$ values for preventing PGE2 formation ex vivo correlated well with antinociceptive activity in mice (0.98) and with in vitro inhibition of brain microsomal cyclooxygenase (0.94). Therefore, this model appears to be predictive of analgesic potency and suggests a central component within this class of drugs.

345.12 EVIDENCE THAT ACUPUNCTURE-LIKE ELECTRICAL STIMULATION OF MOTOR POINTS MODIFIES FUSIMOTOR TONE IN CONTRALATERAL LIMB MUSCLES R.J. Milne, N.J. Dawson*, M.J. Butler* and O.C.J. Lippold, Department of Physiology, University of Auckland, New Zealand.

Electro-acupuncture is one of many physical therapies used to relieve muscle spasm and associated musculo-skeletal pain. Our experiments were designed to determine whether electro-acupuncture is capable of modifying the excitability of spinal reflex pathways, either directly through α motoneurons or indirectly through γ (fusimotor) outflow.

Surface electromyographic (EMG) recordings were made from human extensor digitorum communis (EDC) or first digital interosseous (FDI) muscles. Subjects were instructed to contract the muscle and to maintain a constant level of rectified, integrated EMG. Reflex responses to brief stretch of the muscle, or electrical stimulation of its nerve supply, were extracted from noise by averaging the rectified signal (64 sweeps at 1 second intervals). Reflexes were recorded at 5 minute intervals before, during and after a 25 minute period of acupuncture-like low frequency electrical stimulation of points LI4 (Hoku) and LI11 in the contralateral arm. The cathode was placed proximal.

In one series of experiments, monosynaptic responses to brief stretch of EDC muscle were inhibited by 23% when electro-acupuncture began ($p<0.001$). The effect faded gradually during stimulation. In control experiments, when acupuncture needles were inserted subcutaneously and stimulated with the same current parameters, the reflexes were not affected.

In a second series of experiments, monosynaptic reflexes were elicited alternately in the same subjects by stretch of the FDI muscle or by transdermal electrical stimulation of the ulnar nerve at the wrist. Electro-acupuncture to the contralateral arm depressed the mechanically evoked monosynaptic reflex by 28%, the effect persisting beyond the period of stimulation ($p<0.001$). In contrast, the electrically evoked reflex remained unchanged. Since mechanical stimulation activates muscle spindles whereas electrical stimulation bypasses these structures, we conclude that electro-acupuncture alters somatic reflexes by modifying the sensitivity of muscle spindles to stretch, presumably by depressing fusimotor tone. Such a mechanism could in principle relieve musculo-skeletal pain associated with muscle spasm.

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345.13 AFFERENT PROJECTIONS TO THE MEDIAL THALAMUS Eve Andersen, Dept Anatomy, Univ. Mass Med School, Worcester, Mass

In order to determine the afferent projections to the medial and intralaminar thalamic nuclei (MTh), injections of wheat germ lectin bound horseradish peroxidase (HRP) (2%, 0.1 ul) were made into the following thalamic nuclei: central medial, parafascicularis, central lateral, medialdorsal, centre median and paraventricularis. Rats survived 24-48 hours, and the brains were processed by the tetramethylbenzidine method. Very little, if any injection material was found in the ventral medial, ventral basal, anterior, reuniens, rhomboid, or gelatinous thalamic nuclei, or the habenula.

Retrogradely labeled cell bodies and fibers were found in the brainstem and forebrain. Cell bodies were found in brainstem nuclei associated with the production of neurotransmitters, including the pontine raphe nuclei, midbrain raphe nuclei, ventral PAG, nucleus cuneiformis, locus coeruleus, and substantia nigra. Labeled cell bodies were also found in the somatosensory and reticular activating system, namely in the pontine and midbrain reticular formation, reticular thalamus, zona incerta, and somatosensory cortex. Labeled cells were found in areas associated with motor and posturing functions, including deep layers of the superior colliculus, globus pallidus, ventral pallidum, and entopeduncular nucleus. Labeled fibers, but no cell bodies were found in the caudate nucleus. Finally, cell bodies and fibers were found in areas which process visceral and taste information, the parabrachial nucleus and the deep agranular insular cortex.

There was no apparent labeling in areas of the limbic system, including the septum, hippocampus, hypothalamus, amygdala, entorhinal cortex, or mammillary bodies. There was also no labeling in the motor cortex, medial or lateral geniculate, red nucleus, interpeduncluar nucleus, or trigeminal nuclei. There was no apparent uptake by the fiber pathways which traverse the medial thalamus including the fornix, stria medullaris, mammillothalamic tract, fascicularis retroflexus, or medial lemniscus.

Thus, the MTh receives input from brain areas known to produce modulatory neurotransmitters, and from somatosensory, reticular, visceral, taste, and extrapyramidal motor areas. By far the most heavily labeled areas were the parabrachial nucleus, zona incerta and reticular thalamus. It is very possible that the MTh receives information about visceral and somatosensory pain from these regions. It can also receive modulatory influences from brainstem nuclei which produce neurotransmitters. For example, we have previously shown that the MTh in the rat responds to noxious peripheral stimuli and that this response can be modulated by an ascending serotonergic pain modulation pathway from the dorsal raphe. This evidence suggests that the medial thalamus may have an important role in the modulation of visceral or somatic pain.

345.14 **SUBCOERULEUS-PARABRACHIAL INHIBITION OF CARDIAC INPUT TO SPINOTHALAMIC TRACT NEURONS IN THE MONKEY.** R.D. Foreman, T.J. Brennan*, N.M. Girardot and W.S. Ammons. Dept. of Physiol., Univ. of Okla. HSC, Oklahoma City, OK 73190.

Anatomical studies in the primate have demonstrated that neurons of the subcoeruleus-parabrachial (SC-PB) region project to the dorsal horn of the thoracic spinal cord (Westlund and Coulter, Brain Res. Rev. 2:235, 1980). The purpose of the present study was to examine the effects of electrical stimulation of the SC-PB region on spinothalamic tract (STT) neurons with input from cardiopulmonary (CP) sympathetic afferent fibers of the heart.

Left $T_2$-$T_5$ spinal segments were searched for cells antidromically activated from ventral posterior lateral nucleus and intralaminar nuclei of the contralateral thalamus in α-chloralose anesthetized monkeys. All STT cells received convergent excitatory input from the left chest or arm and the CP sympathetic afferent fibers. Electrical stimulation of the SC-PB region (20-350 μA, 100 Hz, 100 μs) inhibited the background cell activity and the increased discharge rate elicited by noxious pinch of 22 neurons studied. Intracardiac injection of the algesic substance bradykinin excited 8 of 12 cells tested; the responding cells increased their activity from 12 ± 3 to 31 ± 8 spikes/s. Stimulation of the SC-PB region (200 μA, 100 Hz, 100 μs) reduced the peak activity caused by bradykinin to 6 ± 2 spikes/s. The conditioning-test technique revealed that a conditioning stimulus (4 pulses within 10 ms) from the SC-PB region reduced the burst discharge of STT cells (n=5) by 50% about 10 ms after the stimulus was applied to the CP sympathetic fibers. The responses of cells were inhibited for up to 150 ms. Effects of both $A\delta$- and C-fibers were reduced by SC-PB stimulation. Histologic examination showed that effective sites for inhibition were located in the lateral PB, medial PB and SC regions.

In conclusion, electrical stimulation of the SC-PB region produces descending inhibition of visceral input from the heart. In addition, we have shown in earlier studies that stimulation of CP vagal afferent fibers also produces supraspinally mediated inhibition of excitatory input from the heart. In light of anatomical studies in the primate demonstrating that the caudal nucleus of the tractus solitarius receives the vagal afferent input (Beckstead and Norgren, J. Comp. Neurol. 184:455, 1979) and in turn projects directly to the SC-PB region (Beckstead et al., J. Comp. Neurol. 190:259, 1980), the present studies suggest that inhibition of STT neurons by CP vagal afferent stimulation may, in part, be mediated through the SC-PB region. (Supported by NIH grant HL22732, HL27260, and HL07430).

345.15 RECIPROCAL ACTIVITY IN ON- AND OFF-CELLS IN THE ROSTRAL VENTROMEDIAL MEDULLA OF THE RAT. N.M. Barbaro*, H.L. Fields, and M.M. Heinricher. Depts. of Neurosurgery and Physiology and Neurology, University of California, San Francisco, CA 94143.

On- and off-cells are putative pain modulating neurons in the rat rostral ventromedial medulla (RVM). On-cell activity abruptly increases while off-cells pause just prior to the tail-flick response(TF) elicited by noxious heat. In the unstimulated, lightly-anesthetized rat, both on- and off-cells periodically alternate between silent and active periods which last from several seconds to a few minutes. Opiates, whether administered systemically or microinjected into the PAG, produce analgesia and concomitantly abolish the periodic fluctuation in firing: all off-cells become continuously active while all on-cells become silent. Thus, it is possible that the analgesia produced by morphine depends on the synchronization of RVM cells whose periodicity is ordinarily out of phase. The present experiments investigate whether or not the spontaneous activity of all cells within a given class is synchronized in the absence of morphine.

Rats were maintained in a lightly anesthetized state by a continuous infusion of methohexital. The simultaneous activity of pairs of RVM neurons was recorded using two stainless steel microelectrodes placed on opposite sides of the midline near the rostral and caudal poles of the nucleus raphe magnus. A TF-related neuron was found with each electrode, and the spontaneous activity of the pair was recorded.

Twenty-five pairs of neurons were examined in this manner. In every case, two cells of the same class showed synchronous changes in spontaneous activity. In contrast, when the pair consisted of an on- and an off-cell, the two cells showed alternating active periods.

Thus, in lightly-anesthetized rats, all neurons of a given class not only respond in the same way to noxious stimulation and to opiates, but also show tightly coupled fluctuations in spontaneous firing. This phenomenon implies the existence of synaptic connections which synchronize each class of TF-related RVM neurons. These connections could include common external inputs and/or extensive excitatory interconnections among cells of a given class. We have also demonstrated a striking reciprocity of firing between the two cell classes. This could occur if there were a reciprocal input to the two classes, if one class inhibited the other, or if the two classes were mutually inhibitory. Thus, the entire range of opiate effects on RVM neurons could be accounted for by an action on the preexisting synaptic relations between on- and off-cells.

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346.1 FATTY ACIDS EXPAND THE CELL SURFACE AREA OF TISSUE CULTURED NEURONS. H.Horie, Y.Kawasaki* & Takenaka,T. Dept. of Physiol. Sch. of Med. Yokohama City Univ.,Yokohama,232 Japan and Mitsubishi-Kasei Institute of Life Sciences, Tokyo, 194 Japan.

2-Decenoic acid, a fatty acid having 10 carbon atoms, reversibly inhibit the Na-channels of squid giant axons on internal and external application. This fatty acid also increased lateral motion of membrane lipids. This indicates that the fatty acid might be packed in the membrane . Since the axonal surface moves during action potential,it is important for the analysis of the Na-channel inhibition mechanism by the fatty acid to clarify that 2-Decenoic acid changes cell surface area. In this work we have studied effets of 2-Decenoic acid on two aspects of tissue cultured dorsal root ganglion neurons dissected from adult mice. (1) the intensity in a round area, dia 4μm, of fluorescent probe F18, 5(octadecylthiocarbamoylamino)fluorescein, labeled to the neurons (2) size of the neurons measured under Nomarski optics equipped with a computerized video system.

After DRG neurons were incubated in F18 containing medium, only the cell membranes were homogeneously labeled with F18 and the intensity was not affected by changing external solution. It is thought that the intensity is proportional to the concentration of F18 in a unit area of the membrane. When 2.1mM 2-Decenoic acid was externally applied to the labeled neurons, the relative value of the intensity of F18 decreased 0.82 and after washing out the fatty acid the intensity recovered to the same level as before the treatment. It is thought that the intensity might decrease as a result of cell surface expansion caused by the fatty acid and a relative value of the cell surface area might increase about 1.22. From the analysis of the video picture a relative value of the diameter of the neurons increased 1.07 and that of the height of neurons increase 1.12 when 2.1mM 2-Decenoic acid was applied to the neurons. After washing it out this value recovered to the same level as before the treatment. From calculation with these two values, a relative value of the cell surface area increased about 1.20. This value is almost equal to that calculated from the change of the fluorescent intensity. It is thought that the surface membrane expansion might accompany with increase of pressure in the membrane and this pressure might reduce the opening ability of the Na- channels.

346.2 **INITIAL CHARACTERIZATION OF THE INFLUENCE OF SKELETAL MUSCLE (SK) ON THE ELECTRICAL EXCITABILITY OF DORSAL ROOT GANGLION (DRG) NEURONS IN CULTURE.** **G.G. Chen*, A.E. Cole, A.B. MacDermott and J.L. Barker** (SPON: T.G. Smith,Jr.) Lab. of Neurophysiology, NINCDS, NIH, Bethesda, MD 20205.

Mouse DRG neurons maintained in medium conditioned with dissociated cultured SK frequently show repetitive action potential activity (repetitive firing, RF) when injected with sustained depolarizing current. This is in contrast to DRG neurons grown in normal medium which exhibit such behavior far less often (Cole et al., NSA 10:1056, see also for methods). We have examined this action of the muscle-conditioned medium (CM) as well as changes in the excitability of DRG neurons grown in co-culture with SK. Both control and CM-treated cells showed an increased tendency to display RF with increasing age of culture. For example, using whole-cell recording, 16% of control cells and 45% of CM cells at 6-13 days in culture (DIC) show RF while 52% control and 84% CM cells show RF at 22-34 DIC. The increase in frequency of RF occurred when CM was added after as few as 2 or as many as 22 DIC. Young cells were used to test the effect of short duration exposure to CM, since young control DRG neurons showed the lowest level of RF. In experiments with 1.5 to 7 hours of incubation in CM, 15 of 18 cells showed RF while matched control cells had only 1 of 15 cells with RF. Preliminary results suggest that a minimum time of exposure of 30 min is required to elicit RF during continuous application of CM to cells being monitored with repeated depolarizing current pulses. The appearance of RF was not dependent on plating density ($150\text{-}400 \times 10^3$ cells/dish) and was not induced by varying the concentration of NGF (10-200ng/ml). However, the effect on RF could be eliminated by heating the CM at 56° C for 30 min.

We observed that the incidence of RF following DRG neurons co-cultured with muscle was nearly 100%, resulting in a reliable source of cells showing RF. These were used to study the ionic mechanisms driving the action potentials (APs) and ultimately the mechanisms regulating RF. In mouse DRG neurons single APs include both $Na^+$ and $Ca^{2+}$ currents (Ransom & Holz, Brain Res., 136:445, 1977). In our experiments, pressure-applied 1 uM TTX did not block all of the single APs in control cells or first spikes in RF cells, but did block all of the second and subsequent APs in RF cells. These subsequent APs were also more readily blocked by pressure-applied $Na^+$-free medium. Neither the first nor subsequent APs were blocked in the presence of 200 uM $Cd^{2+}$, 2 mM $Co^{2+}$, or 4 mM $Mn^{2+}$. These results indicate that SK releases some factor(s) that is heat labile, not NGF, and requires many minutes (30 min) to induce RF of $Na^+$-dependent APs in DRG neurons.

346.3 **COMPARISON OF THE EFFECTS OF 4-AMINOPYRIDINE (4-AP) AND SKELETAL MUSCLE ON THE EXCITABILITY OF DORSAL ROOT GANGLION (DRG) NEURONS IN CULTURE.** A.E. Cole, G.G. Chen*, A.B. MacDermott and J.L. Barker, Lab. of Neurophysiology, NINCDS, NIH, Bethesda, MD.20205.

We have shown that exposure to conditioned medium (CM) from skeletal muscle (SK) or co-culturing with dissociated SK increases the incidence of repetitive firing in DRG neurons grown in tissue culture (see preceding abstract). In addition, co-culture of DRG neurons with SK nearly doubles the action potential duration of the DRG cells with little change in spike amplitude (n=33). The convulsant 4-AP has been shown to increase cell excitability and prolong the action potential duration in several mammalian neuronal types, perhaps by blocking a transient potassium conductance, the A-current. In the present study, we applied 4-AP (0.5-5mM) to cultured mouse DRG neurons recorded with the whole cell patch technique. In 10/11 control cells, which fired only 1 or 2 action potentials in response to a 100 ms depolarizing pulse, 4-AP produced a rapid increase in the number of action potentials. In addition, the action potential duration was increased in all cells (n=11). In response to long depolarizations (1-2 sec) 4-AP increased cell firing from 1 Hz to 14-20 Hz and showed little adaptation throughout the stimulus. Although there is no evidence at this time for an A-current in DRG neurons, we found that 4-AP blocked a sustained outward current activated by a depolarizing voltage step under voltage-clamp conditions.

It has been suggested that some actions of 4-AP are mediated by an increase in $Ca^{2+}$ conductance (Rogawski and Barker, Br.Res.280:180,1983). To see if these actions of 4-AP on DRG neurons were mediated by an effect on $Ca^{2+}$, we applied 4-AP in the presence of the $Ca^{2+}$ blocker, $Cd^{2+}$ (100-200 uM). Even with $Cd^{2+}$ present, 4-AP still increased cell excitability and prolonged the action potential duration, although the increase in spike duration was not as dramatic. When 4-AP was applied to co-cultured DRG neurons (n=6) that already fired multiple spikes, the number of spikes was decreased, although the action potential duration was prolonged. Neither of these effects was blocked by $Cd^{2+}$.

In the present study, we found that the actions of 4-AP on cultured DRG neurons were similar to the effects of CM or co-culturing DRG cells with SK, including: 1) increased incidence of repetitive firing, 2) prolonged action potential duration and 3) resistance of these actions to blockade by $Cd^{2+}$. Voltage-clamp experiments are in progress to elucidate the mechanism of action of 4-AP and CM on DRG neurons. It may prove useful to compare and contrast the results from these two treatment conditions.

346.4 **WHOLE CELL PATCH AND OPTICAL RECORDINGS OF CALCIUM TRANSIENTS IN SINGLE CLONAL PITUITARY CELLS.** A.B. MacDermott, S.J. Smith*, B. Dufy, J.L. Barker. Lab of Neurophysiology, NINCDS, NIH Bethesda MD 20205.

The present work was undertaken to directly monitor changes in the internal free $Ca^{2+}$ concentration measured by the absorbance of the metallochromic indicator, arsenazo III (Ar III) and the associated electrophysiological events, on single pituitary cells. ArIII (98%, Na salt, Sigma) was dissolved in the recording solution at a concentration of 1-2mM and allowed to diffuse passively into GH3/B6 cells. Patch pipettes contained (in mM): 140 K-gluconate or CsCl, 2 $MgCl_2$, and 5 Hepes. Three photodiodes were used for measurement of transmitted light intensity of GH3 cells at wavelengths centered at 570, 660 and 700 (all ± 15 nm). Large electrical (current steps) and ionic (KCl, 50 mM) depolarizations of the cell membrane evoked peaks in the ArIII signal indicating, as expected, increases in $Ca^{2+}$ cytoplasmic concentration. Currents steps accompanied by multiple action potential firing were also associated with $Ca^{2+}$ signals and at the highest dye concentration, individual spikes evoked individual peaks in ArIII signal (Fig. 1). However, under these conditions, the kinetics of $Ca^{2+}$ removal was slowed. Voltage clamp experiments using CsCl filled electrodes showed that ArIII signals corresponded closely to $Ca^{2+}$ current amplitudes (Fig. 2). Preliminary evidence indicates that under certain conditions (see adjacent abstract), thyrotrophin releasing hormone (TRH) causes an increase in intracellular $Ca^{2+}$ due to $Ca^{2+}$ buffering by the dye.

Fig. 1

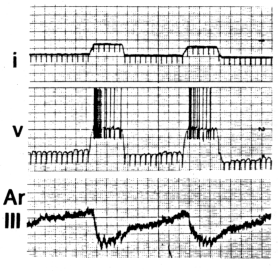
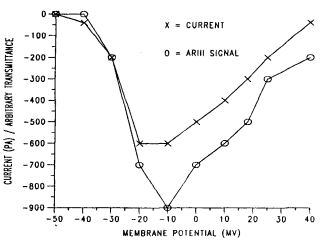



Fig. 2



346.5 A BIOCHEMICAL STUDY OF SINGLE GH3 PITUITARY CELLS USING WHOLE-CELL PATCH RECORDINGS. B.Dufy, A.B.MacDermott, J.L.Barker. Lab of Neurophysiology, NINCDS, Bethesda MD 20205

The stimulus-secretion coupling process by which a secretagogue controls the hormonal release from pituitary cells involves a series of electrical and biochemical events that are not yet completely understood. Electrophysiological events are analysed at the level of the single cell, whereas biochemical processes are normally studied in cell populations. Biochemical studies may be performed on single cells using whole-cell recordings (WCR). WCR technique permits replacing intracellular medium with solutions in the recording pipette while monitoring electrophysiological parameters. We used GH3/B6, a subclone of the GH3 pituitary cell line. The bathing solution consisted of Hanks balanced and buffered salt solution with (in mM) 10 $CaCl_2$ and 2 $MgCl_2$. WRC pipettes had resistances of 3-7 MOhm when filled with the following solution (in mM) 140 K-Gluconate, 2 $MgCl_2$, 5 HEPES, 1.1 EGTA (pH 7.3). Under these conditions the resting potential was -52±9 mV (n=8) and the input resistance was around 3 GOhm. In GH3 cells using conventional intracellular recording techniques with sharp,high resistance microelectrodes, it has been repeatedly reported that thyrotropin releasing hormone (TRH), which stimulates the release of prolactin, induces a transient hyperpolarization of the cell membrane thought to be due to a rapid rise in $Ca_i$ and increase in $K^+$ conductance, followed by generation of action potentials. In the WCR configuration, the effect of TRH (50 nM) vanished very quickly (<5 min) or completely failed to appear in most of the cells recorded (n=35). Restoration of electrical response to TRH was accomplished by filling the WCR pipettes with a cytoplasmic extract of GH3 cells. This was made as follows: $1.5x10^6$ cells were allowed to lyse in the presence of 1 ml distilled water; the supernatant was collected, filtered through a .45 micron millipore filter and then used to prepare the K-Gluconate recording solution. In order to identify the cytoplasmic factor(s) involved in the TRH-induced electrophysiological response, we undertook pharmacological manipulations of the WCR recording solution, including addition of ATP, GTP, and inositol triphosphate (IP3). Only IP3 (5uM) partially substituted for the cytoplasmic extract. At higher concentrations (10-20 uM), IP3 hyperpolarized the cell and decreased input resistance as well as decreasing the amplitude of action potentials. These effects are consistent with persistent high levels of intracellular $Ca^{2+}$. IP3 intracellular concentration may be critical in TRH-induced activation of $K^+$ conductance in GH3 cells but it does not appear to be able to restore the peptide signal to full intensity.

346.6 D-TUBOCURARINE SUPPRESSES SPIKE AFTER-HYPERPOLARIZATION IN GUINEA PIG PREVERTEBRAL NEURONS. N. Mo*, Z.G. JIANG*, N.J. Dun and A.G. Karczmar. Dept. of Pharmacol. Loyola Univ. Med. Ctr., Maywood,IL 60153

Intracellular recordings were made from neurons of the guinea pig celiac-superior mesenteric and inferior mesenteric ganglia in vitro. An action potential evoked by either brief depolarizing current pulse injected through the recording microelectrode or by presynaptic nerve stimulation was followed by an after-hyperpolarization (AH) with an amplitude and duration ranging from 5-25 mV and 30-500 ms, respectively. The AH in most of the neurons clearly exhibited two components; a fast decaying component ($AH_F$) with a time constant ($\tau$) of about 22 ms was followed by a slowly decaying component ($AH_S$) which has a mean $\tau$ of about 85 ms. The $AH_F$ was linearly dependent on membrane potentials and nullified between -90 and -100 mV; the equilibrium potential shifted to a less negative level in solutions of elevated K as predicted by Nernst Equation. $AH_F$ therefore seems to be largely due to an increase of a voltage sensitive K conductance ($G_K$). $AH_S$ appeared to be dependent on extracellular Ca for its generation as it was enhanced by caffeine (5 mM) and eliminated by removing Ca from the bathing solution. $AH_S$ was also membrane potential dependent and nullified at the same potential level as $AH_F$. Hence, $AH_S$ appears to be mediated principally by a Ca activated $G_K$ ($G_{Ca-K}$) as has been suggested for other vertebrate and invertebrate neurons. D-Tubocurarine (d-Tc, 10-100 µM) reversibly suppressed the $AH_S$ in a concentration dependent manner; the half decay time of AH was shortened by 40,55 and 61% in neurons exposed to 10,50 and 100 µM of d-Tc, respectively. The $AH_F$ on the other hand was only slightly depressed by d-Tc. A second type of Ca dependent after-hyperpolarization, namely the post-tetanic (20-30 Hz, 1-2 sec) spike after-hyperpolarization, was also reversibly depressed by d-Tc in a concentration dependent manner. When Na content was replaced with isotonic Ca plus 10 mM tetraethylammonium in the bathing solution, strong depolarizing current pulse evoked in ganglion cells a slowly rising and falling spike potential that appeared to be largely generated by Ca current. Superfusion of d-Tc onto the ganglion cells did not significantly change the amplitude and configuration of the Ca spike potentials. The lack of effect of d-Tc on Ca spike potentials indicates that d-Tc reduced $AH_S$ by interfering with the activation of $G_{Ca-K}$ rather than Ca influx. Collectively, these findings suggest that d-Tc reduced $G_{Ca-K}$, thereby shortening interspike intervals and favoring repetitive cell discharges. Moreover, our results suggest that a shortening of spike AH by d-Tc may contribute to its well known effect as a seizure producing agent in cortical neurons. (Supported by USAMRDC contract DAMD 17-83-C-3133).

346.7 CONTROL OF REPETITIVE FIRING IN RABBIT PARASYMPATHETIC TRACHEAL GANGLION CELLS IN VITRO. J. C. Fowler and D. Weinreich. Dept. of Pharmacology and Experimental Therapeutics, University of Maryland School of Medicine, Baltimore, Maryland 21201.

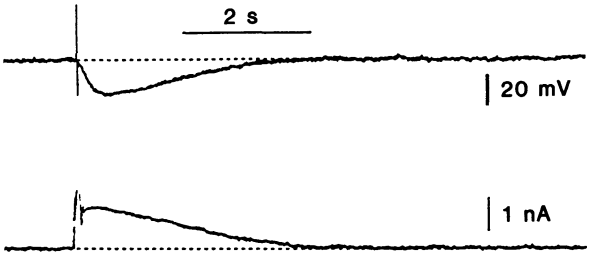
The electrophysiology of rabbit parasympathetic tracheal ganglia was investigated with conventional intracellular recording techniques. Isolated tracheal segments (2-3 cm) were incubated in a .01% neutral red solution for 30 min to visualize ganglia and individual neurons (Skoogh et al., J. Neurosci. Meth. 8:33-39, 1983). The stained ganglia were then undercut and pinned to the floor of a recording chamber maintained at room temperature. Neurons were impaled under visual control at 350 X with glass micropipettes (40-90 MΩ, 1 M KAC). Cells were included in this study only if they showed a stable membrane potential and generated an action potential of greater than 50 mV in response to a depolarizing current step. Values are mean ± S.E.M. These neurons possessed a resting membrane potential of 46.1 ± 2.5 mV (n = 14), an input resistance of 74.0 ± 10.5 MΩ (n = 19) and a time constant of 8.4 ± 1.3 msec (n = 15). Spike height averaged 59.6 ± 3.6 mV (n = 17) with threshold at a membrane potential of 31.7 ± 1.3 mV (n = 17). The fast spike afterhyperpolarization amplitude averaged 14.3 ± 1.2 mV (n = 17) and persisted for 75.5 ± 8.9 msec (n = 11). Slow (several sec) afterhyperpolarizations similar to those observed under comparable conditions in rabbit vagal sensory neurons were not seen (Fowler et al., J. Physiol., in press; Higashi et al., J. Physiol. 355:479-492, 1984).

Neurons could be classified into several types by their response to depolarizing current steps (<1 nA). Some cells fired one (n = 2) or, at most, 4 spikes (n = 6) during a sustained (>600 msec) current step. The remaining cells fired repetitively throughout the depolarizing current pulse (n = 10).

Repetitively firing cells showed a decline in instantaneous firing frequency during a current pulse. Two $K^+$ currents have been shown to modulate firing frequency in peripheral ganglia: a $Ca^{++}$-activated $K^+$ current (Hirst and Spence, Nature 243:54-56, 1973), and an M-current which is reduced by muscarinic agonists and barium ions (Brown et al., J. Aut. Nerv. Sys. 6:23-35, 1982). A substantial role for a $Ca^{++}$-activated $K^+$ current can be excluded: $Cd^{++}$ (100 µM - 1 mM; n = 6), a $Ca^{++}$ current antagonist, and $Ca^{++}$-free (10 mM $Mg^{++}$) Locke solution (n = 1) produced only minimal effects on spike frequency. Carbachol (1-10 µM; n = 2), muscarine (10 µM; n = 1) and acetylcholine (10 µM; n = 1) did not affect spike frequency. However, $Ba^{++}$ (1 mM, n = 2) produced a robust and reversible increase in spike frequency discharge during a depolarizing current step. These results suggest that a $Ba^{++}$-sensitive current which is not affected by muscarinic agonists plays a dominant role in the control of spike frequency in these cells. (Supported by N.I.H. Grant NS 22069 to D.W.)

346.8 HYPERPOLARIZING AFTER-CURRENT IN MYENTERIC NEURONS OF THE GUINEA-PIG ILEUM. T. Tokimasa*, H. Hasuo*, T. Akasu* and R. A. North. *Dept. of Physiol., Kurume Univ. Sch. of Med., Kurume 830, Japan and Neuropharmacology Laboratory, M.I.T., 56-245, Cambridge, MA 02139, USA.

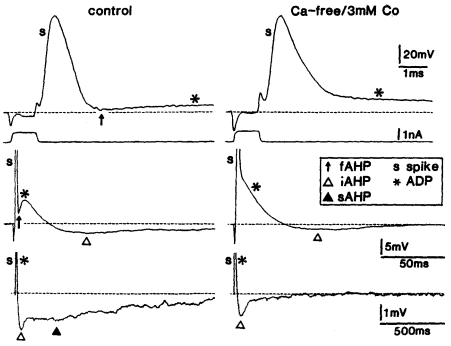
AH (type 2) cells in myenteric plexus of the guinea-pig ileum were voltage-clamped using a single micro-electrode in tetrodotoxin (300 nM) containing Krebs solution (∿ 3 kHz switching frequency with a 50-50 duty cycle). The cells were held at the resting membrane potential of -60 mV and subjected to brief (20-100 ms) depolarizing commands to beyond -25 mV, which evoked a transient inward Ca-current. The inward Ca-current was followed by an outward current during commands. This inward/outward-current was followed by a long-lasting (up to 20 s at -60 mV) outward tail current (see Fig.). The tail current was associated with an increase in membrane conductance. Reversal of the tail current occurred at more negative potential than -90 mV. The tail current became smaller in K-rich (10-20 mM) solution and the reversal potential shifted by about 50 mV for one decade change in extracellular K-concentration. The tail current was markedly augmented in K-free solution and was completely eliminated in Ca-free and Co (1 mM) containing solution. It was therefore concluded that the long-lasting outward tail current arose from an activation of Ca-activated K-current, which corresponds to the spike after-hyperpolarization. The tail current was not blocked by TEA (1-3 mM) or Cs (2 mM) but was markedly depressed by Ba (0.3-10 mM) in a dose dependent fashion. Ba itself mimicked the muscarinic slow synaptic current (North and Tokimasa, J. Physiol., 333: 151, 1982), which was insensitive to TEA (5 mM).



346.9 CALSIUM-DEPENDENT SPIKE REPOLARIZATION, AND THREE KINDS OF AFTER-HYPERPOLARIZATION (AHP) IN HIPPOCAMPAL PYRAMIDAL CELLS. J. Storm*. (SPON: P. R. Adams) Inst. of Neurophysiology, University of Oslo, Norway. Present address: Dept. of Neurobiology & Behavior, SUNY at Stony Brook, Stony Brook NY 11794.

Hippocampal cells are known to have two types of AHP: (1) a slow Ca-dependent AHP (sAHP) lasting 1-5 s, and (2) a shorter (50-100ms) Ca-independent AHP (iAHP). Here I report a third, fast AHP (fAHP, 2-5ms) which follows as a direct continuation of the spike repolarization. Both fAHP and spike repolarization show Ca-dependence and (unlike iAHP and sAHP) are sensitive to low doses of 4-aminopyridine (4-AP) and tetraethylammonium (TEA).

CA1 cells (n=38) were studied in rat hippocampal slices (28-38°C 1-3 mM Ca). Spikes elicited by short (1-2ms) current pulses were each followed by a sequence of afterpotentials: fAHP, an afterdepolarization (ADP), iAHP and sAHP. The same sequence followed each spike during slow repetitive firing (steady current), whereas high-frequency trains of 2-10 spikes were followed by an iAHP and a much enhanced sAHP. Perfusion with Ca-free medium (with 3mM Co, or 10mM Mg/0.5mM EGTA) reversibly slowed the spike repolarization and abolished the fAHP. The sAHP was also blocked, whereas the iAHP and the spike upstroke were essentially unaffected (the spike amplitude increased slightly in some cells). 5mM Mn or 0.2-0.4mM Cd had the same effects. During partial blockade of fAHP, the ADP was often enhanced, and sometimes (n=7) triggered an extra spike. 4-AP (20-100μM) and TEA (1mM) also reversibly slowed spike repolarization and blocked fAHP, but not iAHP or sAHP. These results suggest that a fast Ca-dependent outward current contribute to the spike repolarization in mammalian central neurons, as previously shown in frog ganglion cells.




Ca-dependence of the spike and after-potentials (following a single spike in the same cell) shown at different gains and time-bases. Bottom 4 traces averaged.

346.10 SINGLE CHANNEL CURRENTS RECORDED FROM DENDRITIC MEMBRANES OF CULTURED DISSOCIATED NEURONS FROM THE RAT HIPPOCAMPUS. Leona Masukawa, Anker Hansen* and Gordon Shepherd Section of Neuroanatomy, Yale University School of Medicine, New Haven, CT. 06510.

Previous intracellular studies from isolated apical dendrites of pyramidal neurons from hippocampal brain slices demonstrated that calcium conductances are present principally in dendrites rather than cell bodies (Benardo, Masukawa and Prince, 1982, J. Neurosci 2:1614). These conductances have been postulated to underlie burst potentials during epileptic states.

In order to characterize further the conductances of dendrites, cell attached patch recordings were obtained from apical dendrites (1-3 um in diameter) at sites up to 100 microns from the cell body of cultured hippocampal neurons from 1-2 day old rats. Cells were incubated in DMEM, 10% fetal bovine serum and 2.5% chick embryo extract on collagen coated coverslips for 3-14 days before recordings were made. Single channel current recordings from dendrites during command ramps from rest to 100mV depolarized from rest showed outward currents in control medium. These currents were sensitive to cesium and TEA. The single channel currents increased in amplitude and frequency with depolarizing command voltage steps. These channels were classified as the delayed rectifier ( ~0.6pA and ~12pS) and the Ca-dependent K channel ( ~ 2 pA and ~40 pS) at 60 mV depolarized from rest. Inward currents that were sensitive to cesium and TEA were activated at 40 mV hyperpolarized from rest. These channels were classified as the anomalous rectifier ( ~3 pA and ~150 pS).

In addition, inward single channel currents were present in dendritic cell attached patch recordings during steps from rest to 60mV depolarized from rest. The single channel current amplitudes were ~1pA with a conductance of ~17pS, and were non-inactivating and had short open times, 1-25 msec during 150 msec command steps. These inward currents were classified as Ca-dependent by their insensitivity to TTX (2uM) in the patch pipette. It is hypothesized that these inward single channel currents may underlie the burst response of the dendrite during depolarizing steps and during epileptic states, and that a variety of single channel conductances underlie the regulation of electrical responses in hippocampal neurons.

Supported by NIH grants #NS 10174, #NS 07609 and Weimanns Legat.

346.11 CALCIUM CURRENT IN VOLTAGE-CLAMPED ISOLATED GROWTH CONES. S. Marom* and D. Dagan. Rappaport Family Institute for Research in the Medical Sciences and Department of Physiology and Biophysics, Faculty of Medicine, Technion-Israel Institute of Technology, Haifa 31096, Israel.

Severance of a growth cone from its neurite in primary cultures of Helix aspersa induces rapid withdrawal of filopodia. The isolated growth cone often rolls up spontaneously into a sphere which remains attached to the substrate at one point. The smooth surface of these growth balls and their spherical shape make them ideal geometric bodies for good spatial clamps and, at the same time, enable accurate measurements of the total surface area of any growth cone. Growth cones were obtained from 3- to 6-day-old cultures plated on polylysine- or Helix lectin-treated coverslips. Cells were grown in Leibovitch-15 medium with appropriate salt concentrations at 27°C. Utilizing single-electrode voltage clamping in the whole-cell patch electrode configuration, we measured the ionic currents of isolated growth balls, typically 10-20 μm in diameter.

Membrane current induced by a voltage step from -70 to +20 mV shows an early activating inward current and a delayed outward current when the growth ball is in a medium containing 80 mM NaCl, 4 mM KCl, 7 mM $CaCl_2$, 5 mM $MgCl_2$, 10 mM Tris-HCl, pH 7.4, 10 g/l glucose, and the intracellular solution is dialyzed by the 135 mM KCl electrode solution. Peak inward current is reached in 2-6 ms and inactivates to 50% in 10 ms. Peak outward current is reached in 25-30 ms.

Changing the extracellular solution to one with 10 mM CaCl and 35 mM Tris-HCl, 50 mM TEA, 5 mM CsCl, 5 mM 4-AP, 15 MgCl , and 5 g/l glucose shows an inward current carried by calcium ions. The inward calcium current peaks at +10 mV and reverses at +58 mV. Time to peak of the calcium current in a voltage step from -70 to +20 mV was 3 ms. Mean peak current was found to be 0.3-1.2 pA/μm . This methodology enables us to measure calcium current densities at various conditions during neuronal regeneration.

346.12 PATCH CLAMP STUDIES OF ISOLATED PEPTIDERGIC NERVE TERMINALS. E. Stuenkel*, J. Lemos[1], J. Nordmann* and I. Cooke. Bekesy Laboratory of Neurobiology, University of Hawaii, Honolulu, HI 96822.

The sinus gland (SG), a crustacean neurohemal structure, is a dense aggregation of peptidergic axon terminals. In the crab, Cardisoma carnifex, terminals (5-30 μm dia.) can be isolated by trituration of the SG following collagenase treatment. In EM sections, the isolated terminals appear as circular, membrane-bounded profiles, devoid of adhering material, completely filled with neurosecretory granules (80-210 nm). Each of the terminal types distinguishable morphologically in sections of intact SG are found after isolation. The isolated terminals form 10-40 Gohm seals with electrodes thus permitting patch clamp recording. "Whole terminal" recordings under voltage clamp show both inward and outward currents on step depolarizations from $V_p$ = -55 mV. Inward currents have been resolved into (a) a TTX-insensitive component, rapidly activating, slowly inactivating, that "runs down" after ca. 30 min; and (b) an inward current, activated at steps to $V_p$ = -15 mV or more depolarized that is blocked by TTX and does not run down during recordings lasting 2 hrs. Intracellular recordings from terminals in situ of overshooting action potentials that have both Na- and Ca-dependence permits attributing the currents to Ca (a) and Na (b). Outward current is also activated by steps to $V_p$ = -20 mV or more depol. It shows little inactivation (depol. for >0.5 s), partial block by $[TEA]_o$ = 50 mM, and complete block by internal Cs (from the pipette).

Recordings from inside-out excised patches have been made under conditions favoring K-conductance channels (symmetrical KCl solutions). Two channel types have been observed. One has a slope conductance of ca. 70 pS over a range of $V_m$ = ±55 mV; events occur in bursts at pos. and neg. potentials. Distributions of closed lifetimes are biexponential with bursts and brief (0.1-2 ms) transitions to the open state within the burst; interburst intervals are 0.1-2 s. This type is a K channel, exhibiting rectification on perfusion (internal face) with CsCl or $Na_2SO_4$, but not with $K_2SO_4$ or KCl. A second channel type has a slope conductance of ca. 150 pS. It shows events at both ± $V_m$ having an open state lasting several seconds, with numerous flickers. With $[K^+]_{in}$ high and normal saline in the pipette (high $[Na^+]$ outside), transitions to open state show inward current with steps to negative $V_m$ values, suggesting this channel type is permeable to $Na^+$ as well as $K^+$. Events occur more frequently when $[Ca^{++}]_{in}$ is increased; it is partially blocked by TEA. It thus resembles in ionic characteristics a Ca-activated cation channel. The two channel types are occasionally found in the same patch.

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346.13 EFFECT OF PENTOBARBITAL ON THE TRIPHASIC, PROLONGED CURRENT RESPONSE INDUCED BY CALCIUM INJECTION IN APLYSIA GIANT NEURONS. Jones, L.S., Lewis, D.V., and Wilson, W.A. Duke Univ. Med. Center and Veterans Administration Hospital, Durham, NC 27710.

Following early reports of a monophasic current response to intracellular $Ca^{++}$ injection (Meech, R.W., Comp.Biochem.Physiol. 42A 493, '72), and later reports of a biphasic response (Hofmeier, G. and Lux, H.D., Pflugers Arch. 391:242, '81), the effect of $Ca^{++}$ injections in Aplysia neurons was studied in this laboratory and found to produce a complex, triphasic response (Lewis, D.V. et al., Soc.Neur.Abs. 10:242, '84). When the usually silent R2 or LP1 was held in voltage clamp at resting potential, an iontophoretic injection of $Ca^{++}$ induced a response comprised of an early outward current (phase I) initiated by the injection, followed by an inward current (phase II) that peaked 10-20 seconds after the termination of the injection, then followed by a very slow, prolonged outward current (phase III) that was maximally present 50-100 seconds after the end of the injection. This report examines the results of preliminary studies on the effects of pentobarbital (P) on all three phases of the $Ca^{++}$ induced currents observed.

The triphasic response was most easily seen in cells that were voltage clamped at slightly depolarized potential (-30 to -40 mV) during the iontophoretic injection. The triphasic response induced by $Ca^{++}$ injection was found to be markedly effected by the bath application of 100 uM P. The most obvious effect was a striking, reversible enhancement of the inward current (phase II). Phase II was so enhanced in some cells that it encroached upon the outward phase I, shifting the entire phase I current in a net inward direction. The peak for the phase II current also tended to occur sooner (<15 seconds after the end of the $Ca^{++}$ injection) in P. Where the effect on phase II was very strong, phase III would appear reduced or unchanged, but in cells where the effect on the inward current was less striking, there was evidence that the outward current of phase III also might be enhanced by P. It is possible that the P is routinely enhancing this current but that the effect is being masked by the intensity of the effect on the inward current response. If the outward current of phase III represents the same conductance system as the slow outward current seen in Aplysia giant neurons with depolarization (Zbicz, K.L. and Wilson, W.A., J.Pharm.Exp.Ther., 217:222, '81), then one might expect the P to have the same enhancing effect on phase III that it does on the latter slow outward current.This may be the case.

Preliminary work also shows that removing calcium from the bath doesn't interfere with the triphasic response, and that the P effect on the response is similarly unaffected.

Supported by RO1 NS15212 and the Veterans Administration.

346.14 **CONTROL OF LIGHT EMISSION FROM COELENTERATE CELLS CONTAINING ENDOGENOUS CALCIUM-ACTIVATED PHOTOPROTEIN.** K. Dunlap and P. Brehm. Dept. of Physiology, Tufts Univ. School of Medicine, Boston, MA., 02111.

In the hydrozoan coelenterate Obelia geniculata, bioluminescence is normally triggered by an action potential which propagates through the non-neuronal endoderm. Although light emission requires the presence of extracellular $Ca^{++}$, the nature of coupling between the action potential and the resultant rise in intracellular calcium within the luminescent cells (photocytes) is poorly understood. We studied this interaction by voltage-clamping dissociated endodermal tissue. Following dissociation the photocytes, either in small cell clusters or in isolation, were distinguished from non-luminescent cells on the basis of intense green fluorescence which is characteristic of endogenous calcium-activated photoprotein. Macroscopic membrane currents were recorded by whole cell recording technique from photocytes in artificial sea water (ASW). The electrode contained (in mM) 140 KCL, 11 EGTA, 696 Glucose, 1 $CaCl_2$, and 10 HEPES. All photocytes examined (greater than 60 cells) exhibited a transient outward current which was carried primarily by $K^+$. The reversal potential of the tail current shifted 61 mV per decade shift in external $K^+$ and the current was effectively blocked by inclusion of 1 mM 4-aminopyridine in the external solution. This K channel activated at potentials more positive than -10 mV and inactivated within 150 ms at 20 mV. Both the rate of activation and inactivation increased with depolarization while recovery from inactivation was slowed with depolarization . All of these characteristics were qualitatively similar to those previously described for coelenterate egg (Hagiwara, Yoshida, & Yoshii, J. Physiol.,318:123). Unexpectedly, we never observed a voltage-activated inward Ca or Na current even after block of the transient K current by 4-AP. Moreover, both early and late current-voltage relations were linear both after block of the K current by 4-AP and following inactivation of the K current by depolarization, suggesting that the transient K current is the only voltage dependent current measurable by this technique in photocytes. The lack of voltage-activated Ca current in photocytes was corroborated by the finding that rapid application of ASW containing 360 mM KCl (n=8) or direct electrical stimulation (n=5) did not elicit light in any of the cells examined. On the other hand, small clusters of cells containing one photocyte did elicit light when depolarized with high K (7 out of 8) or when a neighboring non-luminescent cell was electrically stimulated under whole cell clamp (8 out of 8). Light emission was blocked in Ca-free ASW. These data suggest that activation of photoprotein through elevation of internal calcium concentration requires interaction with adjacent non-luminescent cells and that this signal occurs by a mechanism other than direct depolarization. Further experiments are necessary to determine whether synaptic interactions are responsible for control of luminescence from photocytes.

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346.15 Ouabain inhibits the depolarization of frog skeletal muscle induced by palytoxin. Sonia Ortiz* and Gladys Escalona de Motta, Laboratory of Neurobiology and College of Pharmacy, U. of Puerto Rico Med. Sc. Campus, San Juan P.R. 00936.

Palytoxin (PTX) isolated from corals of the genus Palythoa is the most potent marine toxine known. It exerts a $Na^+$- sensitive and tetrodotoxin (TTX)- insensitive depolarizing action on nerves and skeletal muscle cells. It also induces contraction of smooth muscle and cardiac tissues. The toxin purified from P. Caribaeorum (C-PTX) also exhibits a potent hemolytic effect which may be inhibited by ouabain and potentiated by $Ca^{+2}$ (Naunyn-Schmiedeberg's Arch. Pharmacol. 323: 261, 1983). C-PTX fractions were obtained through ethanol (95%) extraction and chromatographic (Amberlite XAD-2) separation monitoring continuosly the toxicity by survival of i.p. injected mice. These fractions depolarized frog sartorius muscle fibers (average $\Delta Vm=80mV$) and caused irreversible contractures of frog rectus abdominus muscles. Both effects were potentiated increasing $[Ca^{+2}]_o$ and were inhibited lowering $[Na^+]_o$. Neither inorganic Ca channel blockers or TTX blocked these C-PTX effects. Ouabain, however, inhibited the muscle responses to C-PTX in a dose and time dependent manner. Ouabain itself exerted a delayed depolarizing action but shorter exposure times (1 hr or less) or lower concentrations (between $10^{-6}$ and $10^{-5}$M) inhibited from 20 to 80% of the depolarizing and contractile actions of C-PTX. These results suggest that the site where C-PTX acts to increase the membrane cation permeability leading to frog muscle depolarization and contraction is related to the $Na^+$-$K^+$ ATPase as it has been proposed to occur in red blood cell and smooth muscles. (Supported by HIH grants RR-0812 and NS-07464. Contribution No. 177 of the Laboratory of Neurobiology.

346.16 ASSOCIATION BETWEEN THE DEPOLARIZING AFTERPOTENTIAL AND THE SUPERNORMAL PERIOD IN MYELINATED FIBERS. C.M. Bowe, J.D. Kocsis,and S.G Waxman. Palo Alto VA Medical Center and Stanford University, Palo Alto, CA 94304

Activity-dependent variations in axonal excitability and conduction velocity have been described for a number of axon systems. Following the relative refractory period, a period of increased excitability, the supernormal period (SNP), is often present. Although the mechanism of the SNP is not well established, early electrophysiologists noted a correspondence of the SNP with the depolarizing afterpotential (DAP) in myelinated axons. Barrett and Barrett (J.Physiol.323:117,1982) have suggested that DAP results from the passive electrical properties of the myelinated axons. However, the direct association of the DAP with threshold changes during the SNP has not been reported. The present experiments were designed to examine the relationship of the SNP to passive membrane depolarization in myelinated fibers.

Sciatic nerves from Wistar rats and frogs were excised and studied in vitro. The nerves were stimulated proximally and compound action potentials were recorded at the distal end. Intra-axonal impalements were obtained with glass microelectrodes (60-120 megohms) filled with 2 M KCl. Eighty-four rat and 67 frog axons having resting potentials >60 mV and spike amplitudes >70 mV were used in this study. Double shock experiments were carried out to evaluate threshold changes following a conditioning stimulus. Eleven per cent of the rat fibers studied at resting potential had an SNP. However, when continuous hyperpolarization was applied to the axon, the DAP increased and 54% of the axons developed an SNP. The SNP began 5-7 msec after the conditioning spike, peaked betweer 10-20 msec, and thresholds gradually returned to control values over 60-120 msec. In addition to whole nerve stimulation, individual axons were stimulated with brief depolarizing pulses applied through the recording microelectrode. Whereas long duration depolarizing pulses produced a period of increased threshold following the pulse, brief depolarizing pulses, even at subthreshold intensities, were capable of eliciting a slowly decaying depolarization after the pulse that resembled a DAP. Furthermore, threshold was reduced during this slow depolarization indicating that the action potential is not a prerequisite for induction of the SNP.

These results support previous observations of the association of DAP with an SNP in myelinated axons. More importantly, the ability of a subthreshold depolarizing pulse to produce both a slowly decaying depolarization similar to the DAP and a concomitant period of reduced threshold similar to the SNP strongly supports the proposal that these phenomena are associated with passive membrane properties in large myelinated fibers.

This work was supported in part by the VA, NIH and the National Multiple Sclerosis Society.

346.17 CHANGES IN MEMBRANE DEPOLARIZATION AND EXTRACELLULAR POTASSIUM CONCENTRATION DURING THE SUPERNORMAL PERIOD OF TURTLE OLFACTORY NERVE. D.L. Eng*and J.D. Kocsis (SPON: M. Miller). Dept of Neurology, Stanford University Medical School and Palo Alto Veterans Administration Medical Center, Palo Alto, California 94304.

The turtle olfactory nerve is a relatively long bundle of homogenous fine caliber nonmyelinated axons. These axons exhibit a pronounced supernormal period (SNP) following a single conditioning volley(Bliss and Rosenberg,Pflügers Arch. 381:209,1979). The SNP is characterized by a period of increased excitability following the relative refractory period. The mechanism of the SNP for nonmyelinated axons is uncertain, but it has been suggested that depolarization associated with accumulation of extracellular potassium, $[K^+]_o$,may account for the SNP(Kocsis et al.,J.Physiol.334,225,1983). To test this proposal we used a combination of the sucrose gap technique with $K^+$-selective microelectrodes to simultaneously monitor membrane potential, $[K^+]_o$, and axonal excitability during the SNP. Adult redear turtles were decapitated and the olfactory nerve was exposed. The nerve was either used in situ or dissected free and placed in a sucrose gap chamber. The nerve was stimulated with stainless steel bipolar electrodes and recordings were made across the sucrose gap chamber and with double barrelled $K^+$-selective microelectrodes. The field potential recorded with a microelectrode following a single stimulus was a triphasic positive-negative-positive wave with a duration of 3.0 msec. However, when recorded across the sucrose gap chamber the return to baseline of the negativity was delayed and occurred with a time course greater than 500 msec. In paired stimulation experiments the excitability of the conditioned response was enhanced as evidenced by latency reduction and increased field potential amplitude. These activity dependent increases in excitability which are indicative of the SNP occurred with a similar time course as the delayed depolarization recorded in the sucrose gap chamber. Additionally an increase in $[K^+]_o$ of about 0.5 mM following a single stimulus occurred with a similar time course as the SNP and the membrane depolarization. Repetitive stimulation led to increases in $[K^+]_o$ to $>$ 7.0 mM, simultaneous to the development of a prolonged membrane depolarization. The excitability of the axons increased during the first several responses of the stimulus train as $[K^+]_o$ began to rise, but was decreased during further stimulation. In order to directly study the effects of $[K^+]_o$ on axonal excitability, the $K^+$ concentration of the superfusate was varied and excitability was tested at the new $[K^+]_o$. As $[K^+]_o$ was increased the resting excitability increased and the SNP was obliterated. Further increases in $[K^+]_o$ led to a reduction in excitability and at $[K^+]_o > 12$ mM the fiber volleys were blocked. These results support the proposal that the SNP in the nonmyelinated axons of the turtle olfactory nerve is the result of activity dependent increases in $[K^+]_o$ that leads to membrane depolarization and a reduction in threshold. Supported by NIH and Med.Res.Ser.ofVA

346.18 POSTSYNAPTICALLY-MEDIATED INCREASES IN EXTRACELLULAR POTASSIUM MODULATE PRESYNAPTIC EXCITABILITY IN HIPPOCAMPUS. N.P. Poolos*, D.L. Eng*, M.D. Mauk, and J.D. Kocsis. Dept. of Neurology, Stanford Univ. Medical School, and Veterans Administration Medical Center, Palo Alto, CA 94306.

Recent studies have indicated that ionic changes in the extracellular space associated with postsynaptic activity, such as accumulation of potassium, can influence the excitability of presynaptic elements (Kocsis et al., J. Physiol 334:225, 1983). We have investigated this effect at the synapse between Schaffer collaterals onto CA1 pyramidal cells in rat hippocampus. Slices of hippocampus (400 um) were stimulated in the stratum radiatum of CA1 with a tungsten electrode. Glass microelectrodes filled with 3 M NaCl were placed 200-400 um away along the longitudinal extent of the Schaffer collaterals to record "on-beam" fiber activation and pyramidal postsynaptic activity. The characteristic field potential following a single stimulus consisted of a short latency negativity of up to 6 mV (N1) corresponding to the presynaptic fiber volley, followed by a smaller negative potential of longer time course (N2), which was abolished in the absence of calcium and thus represented postsynaptic activity. The Schaffer collaterals also showed a supernormal period (SNP), with a submaximal test stimulus evoking a potentiated fiber volley following a single conditioning stimulus. With repetitive stimulation (e.g. 50 hz; 1 sec), the fiber volley and ensuing postsynaptic potential showed a progressive and nearly complete attenuation. This effect is correlated with a rise in $[K^+]_o$: monitoring with K-sensitive electrodes showed that stimulation at 50 hz produced a $[K^+]_o$ rise from a resting value of 3 mM to over 7 mM, which returned to a baseline value with a time course of 2 sec. However, when synaptic transmission was blocked (N2 abolished) by the superfusion of calcium-free Kreb's solution, the presynaptic volley showed little attenuation during repetitive stimulation, and the rise in $[K^+]_o$ was reduced by approx. two-thirds. Similarly, when N2 was abolished by application of adenosine (a putative presynaptic neuromodulator) in normal Kreb's solution at concentrations of 10-100 uM, the fiber volley remained substantially unattenuated and the rise in $[K^+]_o$ was reduced.

These results suggest that activity-dependent rises in $[K^+]_o$ at the Schaffer collateral-CA1 pyramidal cell synapse largely derive from postsynaptic activity, and that these fluctuations in the ionic composition of the extracellular milieu can have pronounced effects upon the excitability of the presynaptic fibers. Since this phenomenon occurs well within the physiological range of hippocampal neuronal firing, extracellular potassium accumulation may prove significant in modulating hippocampal activity, especially during epileptiform discharges.

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346.19 EFFECTS OF PHLORETIN ON NERVE IMPULSE VELOCITY AND MAGNITUDE, AND ON THE STRENGTH-DURATION RELATIONSHIP, IN NONMYELINATED NERVE. E.L. Roberts, Jr.* and D.M. Easton, Department of Biological Science, Florida State University, Tallahassee, FL 32306

Phloretin acts as a blocker of "energy-independent" carrier-mediated ion transport through the cell membrane (cf. Jennings, M.L., and Solomon, A.K., J. Genl. Physiol. 67: 381, 1976). For example, its action on nerve may mimic the defect in Na+-Li+ exchange reported to be present in red blood cells of manic depressives (Tosteson, D.C., Sci. Amer. 244: 164, 1981). We investigated the effects of phloretin on impulse velocity and excitability of the nonmyelinated nerve fibers constituting the olfactory nerve of the longnosed garfish (Lepisosteus osseus). Membrane potentials cannot be measured directly in these small fibers (cf. Easton, D.M. Science, 172: 952, 1971). However, because of the homogeneity of the fibers, dispersion is small, and the external record may be readily interpreted. In a typical experiment, the nerve impulse magnitude and velocity were recorded once every 2 min. for about 10 min. in a control Ringer solution, and then for about 1.5 hr. in a Ringer solution containing phloretin (0-50uM). Strength-duration curves were also determined at the beginning and end of an experiment. Rheobase and membrane time constant were found by fitting to the strength-duration data a modified Blair equation (Blair, H.A., J. Genl. Physiol., 15: 709, 1932). The magnitude and velocity of the nerve impulse and the rheobase of the strength-duration curve decreased, while the membrane time constant increased significantly as the concentration of phloretin was increased. Reversibility of the effects of phloretin was shown by recovery of velocity to better than 90% of its original value following 1 hr. wash after 50uM phloretin. The relative changes in short and long duration limbs of the strength-duration curve and in the early and late phases of the action potential lead us to conclude that phloretin affects both Na+ and K+ conductances, in particular the latter. That conclusion is consistent with the effect of phloretin on the Na+ and K+ ion conductances in the voltage clamped axon of the squid (see Strichartz, G.R., et al., Biophys. J. 31: 229, 1980). (Research support by FSU Computing Center, Psychobiology Research Center, and by a Grant-in-Aid of research from Sigma Xi).

346.20 ELECTRICAL PHENOMENA IN PLANAR MEMBRANES MADE FROM POLYMERS OF AMINO ACIDS.
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Previously it has been shown that spherical cellular structures made from polymers of amino acids (proteinoids) display electrical phenomena. The potential across the membrane and electrical activity has been recorded in such structures both in the presence of phospholipid (Ishima et al., 1981) and in proteinoid-only spherules (Przybylski et al., 1982).

The increase in conductance of the planar cholesterol membranes due to deposition of amino acid polymers has been observed (Grote et al., 1978).

It is known that protein extract (Excitability Inducing Material) deposited on phospholipid membranes increases their conductance and causes spontaneous oscillations as well as membrane potential changes during stimulation (Mueller and Rudin, 1968).

The question of, whether polymers of amino acids would induce similar effects as seen with EIM on planar membranes is being investigated.

Experiments were performed with vegetable phosphatidylcholine dissolved in hexane or octanol using the Mueller-Rudin teflon chamber. Membranes were made across the 1 mm diameter hole. Solutions of the following polymers: p(asp:glu), p(asp:glu:arg), p(asp:glu:pro), p(asp:2 glu: 4 lys: 4 leu: 2 pro) and a proline-rich polymer were added to the central part of the chamber.

The conductance and electrical potential across the membrane were monitored through a M701 WPI amplifier and oscilloscope.

Deposition of the above polymers resulted in a decrease of the membrane resistance and transient appearance of electrical activity. Electrical activity was observed when the membrane resistance reached intermediary values (below $10^6$ and above $10^4$ ohmcm$^2$).

The quantitative difference between the effect of various polymers on the same kind of the phospholipid membrane in varying solvents is under study.

The very low level of electrical features in lipid bilayer vesicles (Mueller and Chien, 1983) as compared to electrical activity across the membrane in the presence of amino acid polymers advances the usefulness of these polymers in studies of the functional structure of the excitable membrane.

347.1 ACTIVITY WHEEL STRESS PRODUCES SEROTONERGIC HYPERSENSITIVITY IN RATS WORKING ON A FOOD REINFORCED OPERANT SCHEDULE. A.R. Mayeda*, J.N. Hingtgen, J.R. Simon, J.S. Gerometta*, P.A. Shea and M.H. Aprison. The Institute of Psychiatric Research and Departments of Psychiatry and Biochemistry, Indiana University School of Medicine, 791 Union Drive, Indianapolis, IN 46223.

Activity wheel stress has been shown in our laboratories to result in a reduction of serotonin (5-HT) in the cortex, hippocampal area, and the midbrain of rats subjected to these chronic stress procedures (Hellhammer et al., Psychosom. Med., 45, 115, 1983). These findings support our hypersensitive serotonergic postsynaptic receptor theory of depression (Aprison et al., In: Neuropharmacol. and Behav., Plenum, p. 23, 1978; Aprison and Hingtgen, In: Serotonin, Plenum, p. 627, 1981). This theory allows us to predict that environmental stress can be a contributing factor in the development of hypersensitive 5-HT receptors. This could occur in two stages: an increased release of 5-HT followed by activation of the autoreceptor system which reduces the level of this release. When the latter is of sufficient duration a hypersensitive system may develop. If this is the case, animals stressed with activity wheel procedures should demonstrate greater sensitivity to 5-hydroxytryptophan (5-HTP) administration (which results in behavior suppression of food reinforced responding) than non-stressed controls. To test this hypothesis, male, Wistar rats were trained to press a lever for milk reinforcement on a variable interval one minute (VI 1) schedule. Following the establishment of baseline responding, rats were then housed continuously in a cage having unrestricted access to an activity wheel. They were given food on a one hr/day schedule and water ad lib. Such procedures lead to the development of symptoms associated with stress (Pare', Brain Res. Bull. Suppl., 5, 73, 1980). Following 7-10 days of activity wheel stress the rats were then given a VI session and received an injection of 25 mg/kg D,L-5-HTP (i.p.) 15 min after the start of the session. This dose of 5-HTP typically results in a very short period (5-10 min) of depressed responding. However, stress pretreatment extended the period of behavioral depression over 5 times that observed in food control rats given the same dose of 5-HTP during a VI session. Serotonin receptor binding studies of specific brain areas obtained from the activity wheel stressed rats are now in progress and should elucidate further the influence of stress on the 5-HTP induced animal model of depression. Using other behavioral methods, Segawa et al. (New Vistas in Depression, Pergamon, p. 3, 1982) and Takahashi et al. (ibid, p. 28, 1983) have already reported finding increased 5-HT receptor sensitivity in stressed rats. (Supported in part by Indiana Department of Mental Health Grant 178-679-005 and by training grant PHS MH 17107-02, NIMH).

347.2 PROLONGED ENHANCEMENT BY SYSTEMIC CHOLECYSTOKININ OCTAPEPTIDE OF HEAD TWITCHING ELICITED BY 5-HYDROXY-L-TRYPTOPHAN IN MICE. K. J. Simansky. Dept. of Pharmacology, Medical College of Pennsylvania, Philadelphia, PA 19129.

Peripheral administration of the sulfonated octapeptide amide of cholecystokin (CCK-8) selectively increased head-twitching but not locomotor activity produced by 5-hydroxy-L-tryptophan (5-HTP) in mice (Simansky, Streisfeld & Friedberg, Neuroscience Abstracts, 1984, 10: 174). The present study examined dose- and time-effect relationships for that phenomenon and compared the action of CCK-8 with those of desulfonated CCK-8 (CCK-DS) and CCK tetrapeptide amide (CCK-4) in the head-twitch assay.

In each experiment, male Swiss-Webster albino mice were injected with carbidopa (25 mg/kg, ip) followed 30 min later by 5-HTP. In the first study, mice (n = 8/group) were also injected just prior to 5-HTP administration with one of the following doses of CCK-8 in 0.3 N $NaHCO_3$ vehicle: 0 (vehicle), 20, 28, 40, 56, 112, 160 or 224 nmol/kg, sc. An observer recorded the number of head twitches made by each mouse during the 5 min period beginning 60 min after 5-HTP (the time of peak effect). CCK-8 increased head twitching in a dose-related manner, $F(7, 56) = 159$, $p < 0.01$. For example, controls made $20.4 \pm 0.6$ head twitches whereas mice that were injected with 28 nmol/kg of CCK-8 made $29.5 \pm 0.6$ ($p < 0.05$) and those given the highest dose made $47.2 \pm 0.9$ ($p < 0.01$). In a subsequent experiment, mice were injected with 160 nmol/kg of CCK-8 either concurrently with 5-HTP or 30 min prior to 5-HTP. Regardless of the pretreatment interval, CCK-8 increased responding by approximately 3-fold during the 2-h test period ($p < 0.01$). The time courses for the behavioral responses to 5-HTP were virtually identical among the four groups (two vehicle- and two CCK-treated sets of mice), with peak effects occurring 60-80 min after 5-HTP.

The enhancement by CCK-8 of head twitching produced by 5-HTP represented a true potentiation because 40 nmol/kg of the peptide failed to elicit that response in mice injected with carbidopa but not with 5-HTP. Furthermore, the excitatory interaction of the peptide and amino acid appeared to be pharmacologically selective because doses of 40, 160 and 640 nmol/kg of CCK-4 and CCK-DS did not significantly increase head twitching during the 5 min test period beginning 60 min after 5-HTP.

Taken together, these data establish that systemically administered CCK-8 potentiates a stereotypic motor response to 5-HTP. Since the head twitch model is an assay for the activation of central 5-HT-2 type receptors, these behavioral data also suggest that CCK-8 causes a persistent increase in the sensitivity or responsiveness of neurochemical systems mediated by 5-HT-2 mechanisms. Alternatively, the potentiation by CCK-8 may reflect a pharmacokinetic interaction between the agents. These issues are are currently being explored. Supported by NIMH Grant MH40574.

347.3 POTENTIATION OF 5-HTP INDUCED DEPRESSION FOLLOWING CHRONIC RESERPINE TREATMENT, K.L. Brugge*, J.N. Hingtgen, & M.H. Aprison, The Inst. of Psychiatric Research and Depts. of Psychiatry & Biochem., Indiana University School of Med., Indianapolis, IN 46223.

As a result of studies begun in the early 1960s, Aprison, Hingtgen and coworkers, proposed a hypothesis concerning the neurochemical basis of depression in a subgroup of patients. Using their model of 5-HTP induced depression in rats and pigeons, they proposed that behavioral depression in animals is caused by an increased release of 5-HT into the synaptic cleft. Since clinical data of depression in man suggest a deficiency rather than an excess of 5-HT, they reconciled what at first appeared opposing views and hypothesized that in some types of depression, hypersensitive postsynaptic receptors develop after a period of prolonged reduction in 5-HT release. This hypothesis is supported by Fleisher et al. (J. Neurochem. 32, 1613, 1979) who showed potentiation of the 5-HTP induced behavioral depression in rats along with increased receptor affinity of ($^3$H)-5-HT in crude membrane fractions from the cerebral cortex of these animals, following chronic p-chlorophenylalanine (PCPA) treatment. Since reserpine is known to precipitate depression in up to 26% of hypertensive patients receiving it chronically, the present experiment was designed to determine whether the effects of chronic reserpine on 5-HTP induced behavioral depression were similar to that seen after PCPA. Because reserpine acts by reducing storage of monoamines, thereby causing their depletion, we predicted the development of receptor supersensitivity, including the 5-HT receptors, with a resulting potentiation of the 5-HTP induced depression. Male Wistar adult rats were trained to barpress for milk on a VI 1 reinforcement schedule. Once baseline responding was established, the rats were given a threshold dose of 25 mg/kg 5-HTP (i.p.) 15 min. after the start of a VI session. The rats were then subdivided into 3 treatment groups. Two groups were started on daily i.p. injections of 0.05 mg/kg or 0.025 mg/kg reserpine, and the third group on daily vehicle. Most of the rats of both the 0.05 mg/kg and 0.025 mg/kg treatment groups showed a significant potentiation of 5-HTP induced depression after 6 days of chronic treatment with reserpine. Three rats, however, failed to show potentiation after 6 and 13 days of treatment. When the daily reserpine dose was increased to 0.10 mg/kg, these rats also showed increased depression after a 25 mg/kg injection of 5-HTP. These data suggest that chronic reserpine produces similar effects to those of chronic PCPA thus supporting a biochemical-behavioral animal model of depression involving the serotonergic system. (Supported in part by Ind. Dept. of Mental Health Grant 178-679-005 and in part by the Assoc. for Adv. of Mental Health Research and Education, Inc., Indianapolis).

347.4 THE PIPERAZINE SEROTONIN (5HT) AGONISTS m-CHLOROPHENYLPIPERAZINE (m-CPP) AND 6-CHLORO-2(1-PIPERAZINYL)PYRAZINE (MK 212) IMPAIR AVOIDANCE RESPONDING IN A DISCRETE TRIAL CONDITIONED AVOIDANCE PROCEDURE. J.A. Moyer and I. Lucki, Wyeth Laboratories, Inc., P.O. Box 8299, Philadelphia, PA 19101 and Department of Psychiatry, University of Pennsylvania, Philadelphia, PA 19104.

Serotonergic neuronal systems have been implicated in the mediation of aversive operant behavior; however, the effects of substituted piperazine 5HT agonists on conditioned avoidance performance have not been clearly defined. Therefore, the effects of the potent piperazine 5HT agonists MK 212 and m-CPP were examined in a discrete trial/lever press conditioned avoidance procedure.

Male rats were trained to avoid electric shock during trials consisting of a 15 sec warning tone (conditioned stimulus) followed by a 15 sec tone/shock period (unconditioned stimulus). A response (lever press) during the initial 15 sec period terminated the trial prior to shock delivery and was considered an avoidance response. A response which occurred during the tone/shock period was considered an escape response. Trials were presented on a variable interval schedule of 2 min and each session consisted of 60 trials. Animals were run 2-3 times weekly with control sessions always preceding drug runs.

MK 212 and m-CPP, administered i.p. 30 min prior to the test session, suppressed avoidance responding in the discrete trial conditioned avoidance procedure (avoidance block $_{50}$ = 1.46 and 3.92 mg/kg respectively). Furthermore, both of these 5HT agonists produced increases in escape responding and the ability to escape from shock was not altered by the doses used in these experiments. Additional experiments determined that pretreatment with the 5HT antagonist metergoline (2 mg/kg i.p., 90 min prior to test session) blocked both the impairment of avoidance and increase in escape responding produced by MK 212 and m-CPP. However, metergoline pretreatment did not block reductions in avoidance responding and increases in escape behavior produced by haloperidol (.25, .5, and 1 mg/kg i.p.). Metergoline and ketanserin ($5HT_2$ antagonist) had no significant effect on either avoidance or escape performance when administered alone.

The results of these studies show that the piperazine 5HT agonists MK 212 and m-CPP produce a behavioral pattern similar to antipsychotic compounds in a discrete trial conditioned avoidance procedure. However, since metergoline antagonized the effects of 5HT agonists on conditioned avoidance behavior, but not the effects of the dopamine antagonist haloperidol, 5HT agonists may impair conditioned avoidance through mechanisms which are different from the antipsychotics.
Supported in part by USPHS Grant MH-36262.

347.5 ANTICONFLICT EFFECTS OF THREE COMPOUNDS HAVING ANTISEROTONERGIC ACTIVITY: METERGOLINE, MIANSERIN, AND CLOZAPINE. J.W. Sullivan*, L. Gold*, and J. Sepinwall. Dept. Pharmacology and Chemotherapeutics, Hoffmann-La Roche Inc., Nutley, NJ 07110.

One pharmacologic characteristic of the benzodiazepine class of drugs is their ability to release - or disinhibit - behavior that is suppressed by punishment. Several reports have indicated that antiserotonergic substances also do this, and one recent report (Boast et al., Soc. for Neurosci. Abstr., 9:436, 1983) demonstrated a positive correlation between antipunishment effects and anti-serotonergic activity.

We evaluated two serotonin antagonists (metergoline and mianserin) and one antipsychotic compound with some reported antiserotonergic properties (clozapine) in rats in a 47 minute long multiple schedule conflict test comprised of alternating components of unpunished (VI30 sec. food; 5 min.) and punished (FR10 food + shock; 2 min.) responding. Metergoline ( 2.5-20 mg/kg p.o.), clozapine (5 mg/kg p.o.), and mianserin ( 0.5-7.5 mg/kg i.p.) produced statistically significant (dose-related) increases in punished response rates. All three compounds had inverted U-shaped dose-response profiles, with clear signs of non-specific rate-depressant effects occurring at the upper end of the effective dose range.

We attempted to block the anticonflict effect of metergoline by administering Ro 15-3505, an imidazobenzodiazepine derivative related to Ro 15-1788, that binds to the benzodiazepine receptor and antagonizes the pharmacologic effects of benzodiazepines. When given alone (1.25-40 mg/kg p.o.), Ro 15-3505 was devoid of any antipunishment properties but did decrease unpunished response rates. In drug combination studies, Ro 15-3505 (5 mg/kg) failed to block the anticonflict effect of 20 mg/kg of metergoline, whereas it did block the anticonflict effect of 10 mg/kg of chlordiazepoxide. Thus, compounds having serotonin antagonistic activity exert their effects at sites different from the benzodiazepines.

347.6 DISRUPTIVE EFFECT OF 5-METHOXY-N,N-DIMETHYLTRYPTAMINE (5MeODMT) ON SHUTTLEBOX CONTINUOUS AVOIDANCE BEHAVIOR IN RATS: EFFECTS OF VARIOUS BLOCKERS OF SEROTONIN (5HT) RECEPTORS. F.P. Miller, Merrell Dow Research Institute, Cincinnati, OH 45215

In rats, 5MeODMT causes a complex syndrome characterized by the occurrence of at least six discernible symptoms, some of which can be antagonized by 5HT antagonists (Jacobs, B.L., Life Sci. 19:777, 1976). The scoring of such a behavioral syndrome is highly subjective. Therefore, we investigated the use of shuttlebox continuous avoidance behavior as an objective method of assessing the response of rats to 5MeODMT.

Rats were trained on a 40 sec RS-20 sec SS schedule to avoid an electric shock through a grid floor by moving back and forth across the center of a shuttlebox. Rats trained to avoid $\geq$ 95% of the possible shocks were administered various doses of 5MeODMT, and behavior (avoidance, shocks, escape failures) was automatically recorded for 5 hours following administration with behavioral changes being evaluated hourly compared to vehicle-treated control. At doses of 1, 2 and 4 mg/kg i.p., no significant effect was seen in avoidance rate (n=4), even though at 4 mg/kg, slight signs of the 5MeODMT-induced syndrome were observed visually. Mean changes in avoidance rates, compared to vehicle controls, were -6.2%, -3.6% and -18.1%, respectively. At 5.0 mg/ kg i.p., marked disruption of avoidance behavior was seen (-43.7% compared to vehicle control during the first hour after administration), concomitant with marked symptoms of 5MeODMT-induced syndrome; these effects lasted for 15-30 min in each animal.

The avoidance deficit induced by 5MeODMT, 5 mg/kg i.p., was completely antagonized by methysergide, a known 5HT antagonist, in doses as low as 0.25 mg/kg i.p. given 30 min prior to 5MeODMT. Thus, first hour avoidance rates after 5MeODMT in animals (n=4) pretreated with methysergide at 0.25, 0.5, 1, 2 and 4 mg/kg i.p., were +21.1%, +15.1%, +21.3%, +29.2% and +57.2%, respectively, compared to vehicle controls; at 0.125 mg/kg i.p., methysergide was ineffective. Metergoline was similarly effective at doses $\geq$ 0.25 mg/kg i.p. Cyproheptadine, tested at 0.5, 1, 2, and 4 mg/kg i.p., caused antagonism (avoidance rate +1.2% compared to vehicle control) only at 2.0 mg/kg. Pipamperone was ineffective at 1 or 2 mg/kg i.p.

These results with methysergide, metergoline, and pipamperone are in substantial agreement with those reported by Lucki et al. (J. Pharmacol. Exp. Ther. 228:133, 1984) using the syndrome response elicited by 5MeODMT in rats; cyproheptadine was not examined in their studies. Further, these investigators have suggested that the behavioral response to 5MeODMT probably involves $5HT_1$ receptors. Therefore, our results in the continuous avoidance model suggest that this model provides an objective method of assessing the activities of drugs interacting with $5HT_1$ receptors.

347.7 EVIDENCE THAT SEROTONIN MEDIATES NON-CHOLINERGIC ELECTROCORTICAL ACTIVATION AND COGNITIVE ABILITIES. C. H. Vanderwolf and G. B. Baker. Dept. of Psychology, Univ. Western Ontario, London, Ontario, Canada, N6A 5C2 and Neurochemical Research Unit, Dept. of Psychiatry, Univ. Alberta, Edmonton, Alberta, Canada, T6G 2G3.

Previous research has shown that low voltage fast activity (LVFA) in the neocortex and rhythmical slow activity (RSA) in the hippocampus can result from activity in either of two ascending pathways. Activity in neurons in the basal forebrain (substantia innominata, medial septum, diagonal band) may produce atropine-sensitive (presumably cholinergic) LVFA and RSA during both Type 1 behavior (e.g., head movement, walking) and Type 2 behavior (e.g., waking immobility, face-washing, tremor). Activity in an aminergic pathway may produce atropine-resistant LVFA and RSA during Type 1 behavior only (Stewart, D. J., et al., Brain Research, 322: 219, 1984; Vanderwolf, C. H. & Robinson, T. E., Behav. Brain Sci., 4: 459, 1981). The possible role of 5-hydroxytryptamine (5-HT) in such a pathway was studied by treating rats with p-chlorophenylalanine (PCPA; 500 mg/kg, i.p.) on each of 3 successive days. Assays using HPLC and electrochemical detection indicated that 5-HT levels in the neocortex and hippocampus were reduced by 93% of normal on the fourth day while noradrenalin was reduced by 33% and dopamine by 23%. Hippocampal RSA was quantified by passage through a band-pass filter (6-12 Hz) followed by rectification and integration. Neocortical activity was scored manually in successive 1 sec intervals. Multiunit activity was also monitored in the neocortex. PCPA treatment alone had little effect on LVFA or RSA, but following PCPA and atropine (50 mg/kg) together both LVFA and RSA were attenuated or eliminated, resulting in continuous large amplitude irregular activity in the hippocampus and large irregular slow waves in the neocortex regardless of concurrent behavior. Thus, atropine-resistant LVFA and RSA may be dependent on 5-HT transmission. Tests in a water maze showed that while atropine alone or PCPA alone produced slight impairments in goal-directed behavior, the two drugs together produced a massive deficit. A combination of PCPA and atropine may produce an animal model of some forms of global human dementia.

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347.8 AGGRESSION IN WEAKLY ELECTRIC FISH - A SENSITIVE ASSAY FOR THE PHARMACOLOGY OF MONOAMINERGIC NEUROTRANSMISSION. L. Maler and W. Ellis*. Dept. of Anatomy, University of Ottawa, Ottawa, Ontario K1H 8M5 Canada.

Agonistic behaviour is a complex combination of threat signals and overt aggressive action. Weakly electric fish offer a technically simple preparation which still retains the critical features of vertebrate aggression. Apteronotus leptorhynchus (Brown Ghost knifefish) is a South American gymnotid fish. Apteronotids produce a constant high frequency (600-1000Hz) electric organ discharge (EOD) and detect their own and conspecifics EODs with specialized electroreceptors distributed over their body. Apteronotids modulate their EOD in several ways, the two most prominent being: (1) the jamming avoidance response (JAR) during which an apteronotus will shift his EOD frequency upwards away from that of a conspecific 1Hz to 10Hz lower in frequency, and (2) a brief (<100msec.) increase in frequency (5-80Hz) accompanied by a decreased amplitude of the EOD. This latter behaviour has been named a "chirp" and, because it accompanies aggressive behaviour like biting, is believed to be an aggressive signal. We curarize the fish, expose the forebrain ventricle and maintain the fish with artificial respiration for several hours. Another fish is simulated by a pair of transversely oriented carbon electrodes. We use these electrodes to produce a sine wave with a frequency 3Hz below that of the experimental animal and an amplitude of 2.5mv/cm (measured at the head); a stimulus regime of 30sec. on and 5min. off is used. Under these conditions both normal JARs and chirps can be elicited. Minute doses of serotonin (0.1µg) strongly inhibit chirping, an action consistent with evidence from mammalian species that this transmitter inhibits agression; 5-HT has no effect on the JAR indicating that sensorimotor processing is not globally affected. The same dose (0.1µg) of noradrenaline strongly enhances chirping but again has no effect on the JAR. Dopamine has more complex effects on chirping, with initial excitation sometimes followed by inhibition and also briefly enhances the JAR. Neither tryptamine nor the artificial CSF+ ascorbic acid carrier have any effect on chirping. This behaviour is easy to elicit, completely quantifiable and so sensitive to monoamines that monoamine oxidase inhibitors are not required to get these effects. Accordingly we have used this preparation to investigate several drugs believed to act on monoaminergic transmission.

Supported by the Medical Research Council of Canada.

347.9 EFFECTS OF 5-HYDROXYTRYPTOPHAN ON SCHEDULE-CONTROLLED BEHAVIOR: EVIDENCE FOR PERIPHERAL MEDIATION BY 5-$HT_1$ RECEPTORS. R.B. Carter, Dept. of Pharmacology, Georgetown Univ., Washington, DC 20007.

Recent evidence has emphasized the contribution of peripheral mechanisms to the behavioral and physiological effects observed following systemic administration of 5-hydroxytryptophan (5-HTP) (Neuropharmacology 19: 777-784, 1982; Pharmacol. Biochem. Behav. 9: 249-253, 1978; 20: 799-802, 1984). While these data suggest that the effects of 5-HTP are due to the actions of one of its decarboxylated metabolites in the periphery, they do not necessarily indicate that these effects are mediated entirely through serotonergic receptors. The effects of l-5-HTP (1.0-30.0 mg/kg, i.m.) on the responding of pigeons maintained under a multiple FR 30, FI 5-min schedule of food presentation were studied alone and in combination with the uptake blockers fluoxetine (10.0 mg/kg, i.m.), citalopram (5.0 mg/kg, i.m.) and nisoxetine (0.3 mg/kg, i.m.), the antagonists methiothepin, metergoline and LY53857 (0.01-1.0 mg/kg, i.m.) and the peripheral decarboxylase inhibitor benserazide (25.0-75.0 mg/kg, i.m.). l-5-HTP alone produced only dose-related decreases in responding under each schedule component. Fluoxetine, citalopram and nisoxetine failed to alter responding when given alone. When administered in combination, the serotonin (5-HT) uptake blockers fluoxetine and citalopram, but not the norepinepherine (NE) uptake blocker nisoxetine shifted dose-response curves for l-5-HTP to the left by a factor of 3-10. The 5-$HT_1$/5-$HT_2$, NE and dopamine antagonist methiothepin, given alone did not alter responding under the FR component, but produced dose-related decreases in FI responding. When administered in combination, methiothepin shifted dose-response curves for l-5-HTP to the right in a dose-dependent manner. The degree of shift in the FI component was limited by methiothepin's own rate-decreasing effects. Metergoline alone either did not alter or produced slight increases in responding under both components. In combination, the 5-$HT_1$/5-$HT_2$ antagonist metergoline shifted curves for l-5-HTP to the right in a dose-dependent manner. LY53857 alone either did not alter or increased rates of responding under both components. When administered in combination, the specific 5-$HT_2$ antagonist LY53857 failed to shift dose-response curves for l-5-HTP to the right, although antagonism of the effects of a low dose of l-5-HTP did occur. The peripheral decarboxylase inhibitor benserazide (25.0-75.0 mg/kg), was also studied in combination with l-5-HTP. Benserazide attenuated the effects of 30.0 mg/kg l-5-HTP in a non-dose-dependent manner. Taken together, these data suggest that the effects of high doses of l-5-HTP on schedule-controlled behavior in the pigeon are due to actions of serotonin on 5-$HT_1$ receptors outside of the blood-brain barrier. These data extend previous findings with regard to the role of peripheral mechanisms in the behavioral effects of 5-HTP to a nonmammalian species. (MH-14269 & DA-05253).

## BEHAVIORAL PHARMACOLOGY: ADRENERGIC AND HISTAMINERGIC SYSTEMS

348.1 **BEHAVIOURAL AND PHYSIOLOGICAL RESPONSES TO ACUTE TREATMENT OF AN ALPHA-ADRENERGIC AGONIST--FLA-136.** S. M. Socaranksy*, R. Vanier*, Z. Amit, S-O Ogren. Dept. of Psychology, Ctr. for Studies in Behavioural Neurobiology, Concordia University, Montreal, Canada and Astra Pharmaceutical Research Ctr., Sodertalje, Sweden.

We have previously shown that treatment with the clonidine analog FLA-136(4-amino-3-(2,6-dichloro-benzlidene-hydrazino)-1,2,4-triazole hydrocloride) can significantly suppress voluntary ethanol administration on a chronic basis. Presented here is a series of studies which set about trying to elucidate the mechanism by which such an effect may occur. In the first study effects on motor activity were investigated in rats treated with FLA-136 alone, or in addition to ethanol (.6, 1.2g/kg). FLA-136 was administered at a dose previously shown to maximally reduce ethanol intake (25 mg/kg). Motor acitivity was measured in boxes equiped with photoelectric cells attached to automated counters, the total number of counts being determined for a thirty minute period. Brain and blood samples were taken at the end of this period for determination of ethanol and, NE and MHPG levels. It was found that while FLA-136 reduced locomotor activity in and of itself it also potentiated ethanol mediated suppression of activity. These findings tended to be consistent with brain MHPG levels is each of the different groups. In the second experiment, FLA-136 was used as a conditioning drug in a conditioned taste aversion paradigm (CTA) whereby doses (15, 25 mg/kg) where administered to rats immediately after having consumed a novel solution. FLA-136 elicited an extremely reliable CTA at both doses employed. In the third experiment the effects of pretreatment with FLA-136 on an ethanol induced CTA was investigated. In this paradigm FLA-136 (15, 25 mg/kg) was administered four hours prior to the pairing of the presentation of the novel solution with administration of various doses of ethanol (1.2, 2.0 g/kg). The results of this latter study point to a potentiation of the ethanol mediated conditioned taste aversion by pretreatment with FLA-136. The sum of these findings would seem to suggest that some form of potentiation or summation exists between the down regulation of NE turnover by FLA-136 and the effects of ethanol on these same systems as evidenced by the behavioural and the biochemical data presented here.

348.2 STUDIES ON THE REINFORCING PROPERTIES OF CLONIDINE IN RATS. K.E. Asin and D. Wirtshafter. Department of Psychology, Univ. Il. at Chicago, Chicago, IL 60680.

We have recently reported (Asin & Wirtshafter, Psychopharm., 1985) that clonidine (CLON) produces a conditioned place preference (CPP) in rats. This effect was dose-dependent and was only seen with doses which have been shown to affect both $\alpha_1$ & $\alpha_2$ receptors (200 & 400ug/kg ip). This series of studies investigated the neurochemical bases of the CLON-induced CPP and also examined the effects of the drug on the establishment of a conditioned taste aversion (CTA).

Place preference conditioning was conducted in shuttleboxes with 1 end painted grey and the other painted with black & white stripes. Animals were randomly chosen to receive drug prior to confinement in the striped or grey side; vehicle (V) injections were paired with the side opposite to that of the drug. Rats were given 5 drug/stimuli pairings and 5 V/stimuli pairings. On the test day, rats were injected with V and were allowed to traverse the entire apparatus for 15min. Time on the previously drug-paired side was taken as an index of reinforcement and CPP (chance=450s). A dose of 400 ug/kg CLON, injected 3min before placement in the apparatus, was chosen for the CPP studies.

Injections of the mixed $\alpha_1/\alpha_2$ receptor antagonist phentolamine (5,10&25 mg/kg) 45min prior to CLON treatment failed to block the establishement of a CPP. Similarly, rats treated with 2 or 5 mg/kg (ip) of the $\alpha_2$ antagonist yohimbine 20min prior to CLON injections showed CPPs similar in magnitude to V+CLON treated animals. Injections of the $\alpha_1$ antagonist prazosin (.05,.1,2.5 & 5mg/kg) 20min before CLON also failed to affect the magnitude of the CPP compared to V+CLON treated rats. These results suggest that some non-noradrenergic property of CLON may underly its reinforcement efficacy. This conclusion is further supported by our failure to obtain a significant CPP using the $\alpha_2$-receptor agonist guanfacine (1, 2 & 4 mg/kg ip). Therefore, we also examined the effects of pretreatment with the opiate antagonist naloxone (2&6mg/kg, 10min pre-CLON) or the dopamine antagonist haloperidol (.3&.6mg/kg ip)(45min prior to CLON) on a CLON-induced CPP. Neither of these drugs was able to attenuate or block the CPP. These studies suggest that animals may find CLON's histaminic properties or its effects on brain epinephrine systems reinforcing. Additional studies using drugs with more specific $\alpha_1/\alpha_2$ properties will also be presented.

Finally, we examined the effects of CLON (0,50,100,200 & 400 ug/kg ip) on the establishment of a CTA to a sucrose solution in thirsty rats. CLON was found to produce a dose-dependent CTA in both 1 & 2 bottle preference tests. Thus, similar to what has been reported for drugs of abuse, such as amphetamine or morphine, CLON has both reinforcing and aversive properties. The pharmacological profile of these properties, however, remains to be determined.

348.3 SPINAL CORD NOREPINEPHRINE, NOCICEPTION AND CLONIDINE ANALGESIA: SEX DIFFERENCES? B. A. Pappas and R. Ings*. Psychology Dept., Carleton University, Ottawa, Canada K1S 5B6.

Evidence suggests a descending norepinephrine (NE) spinal pathway of supraspinal origin which inhibits nociception at the spinal level. Previous research from this laboratory has shown that this NE pathway is permanently lesioned by neonatal intraspinal injection of 6-hydroxydopamine (6-OHDA) and that this lesion, as expected, enhanced nociception as assessed by the tail flick task. Unexpectedly, however, this effect occurred in female but not male rats. This experiment reexamined this sexual dimorphism and also determined if the analgesic effect of the alpha NE agonist clonidine and the binding of clonidine to the spinal cord were altered by NE lesion in a sexually dimorphic manner.

Intraspinal 6-OHDA (10 µg) was administered to rats on days one and two of life. At about 120 days of age, nociception was assessed using the tail flick (tail lowered into 54°C water). Males (intraspinal vehicle or 6-OHDA injected) were found to have longer latencies than their corresponding females. This sex difference may be due to circulating testosterone as castration of otherwise normal males lowered their tail flick latencies. Conversely, ovariectomy did not affect female latencies.

In agreement with our earlier results, intraspinal 6-OHDA lowered latencies in females but not males. The analgesic effect of clonidine (1 mg/kg) was not eliminated but rather was augmented by the 6-OHDA lesion. This occurred only in females, however, suggesting that at least for them, behavioral supersensitivity develops in the spinal cord after the loss of NE terminals. This sex difference cannot be accounted for by differential response to 6-OHDA since both males and females showed massive losses of spinal NE (82 and 91%, respectively). Furthermore, it cannot be explained by differences in spinal alpha receptor proliferation after 6-OHDA. Receptor binding assay using ($^3$H) clonidine as ligand showed that 6-OHDA did not affect spinal clonidine binding in either sex. $B_{max}$'s for control males and females were 84.6 + 9.2 and 80.5 + 12.9, respectively, while for the comparable 6-OHDA groups they were 75.5 + 13.8 and 86.6 + 10.3. Parenthetically, these data indicate that as has been found for the cerebral cortex, the alpha 2 receptor in the spinal cord does not appear to be affixed to the NE terminal.

In conclusion, the antinociceptive role of spinal NE is more apparent in female than male rats. Second, neither clonidine analgesia nor the binding of this drug to the spinal cord requires intact NE terminals. (Supported by NSERC)

348.4 CEREBRAL DEPLETION OF NOREPINEPHRINE WITH DSP-4 FAILS TO ALTER ACQUISITION OF A CONDITIONED AVOIDANCE RESPONSE IN TWO STRAINS OF RAT. R. J. Elgin, Jr.* and G.E. Martin. Department of Biological Research, McNeil Pharmaceutical, Spring House, PA 19477-0776.

The ability to block conditioned avoidance responding (CAR) is a property of all known antipsychotic drugs. To examine the role of cerebral norepinephrine (NE) in mediating CAR, the effect of DSP-4, an agent known to deplete cerebral NE (Ross, B.J. Phar. 1976,58:520), on the acquisition of a CAR response was studied in two strains of rat, Fisher (F) and Sprague-Dawley (S/D).

DSP-4(N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine), in a dose (50 mg/kg,i.p.) which produces maximal depletion of cerebral NE, was given to groups of 10-18 rats from each strain. In one control group, desmethylimipramine (DMI)(20 mg/kg,i.p.-30 min) which inhibits DSP-4's uptake was given prior to DSP-4. There were 4 treatment groups: 1) saline-saline; 2) saline-DSP-4; 3) DMI-DSP-4; 4) DMI-saline. Starting 10 days after treatment, all animals were tested for acquisition of CAR, in which a discrete trial lever press avoided a 0.7 mA shock. A test session consisted of 120 trials spaced evenly over a 1 hr period. The conditioned stimuli (paired light and tone) were presented for 15 sec followed by 10 sec of shock in the absence of a lever press. Each animal was tested once daily for nine consecutive days. Performance on each day was compared between groups using analysis of variance with Duncan's multiple range test ($p<0.05$).

Rats were sacrificed 20 days after dosing and the cerebral cortex, striatum, and brainstem were rapidly dissected on ice and homogenized for assay of NE, and dopamine (DA) levels using an HPLC with electrochemical detection. To examine DSP-4's effect on trained rats, DSP-4 or saline was given to trained rats and CAR was observed 10 days later for 4 consecutive days.

In each strain, DSP-4 produced an almost complete depletion of NE in the cortex and ca. a 50% reduction relative to control groups 1,3, and 4 in the brainstem, whereas striatal dopamine levels were unaltered. In the F strain DSP-4 clearly failed to alter CAR performance during the 9 day acquisition period. In the S/D strain there was an apparent impairment in acquiring the CAR task, although a statistically significant difference from the DMI-saline control group was the only difference detected. Furthermore, DSP-4 given to trained rats of either strain, caused no decrement in CAR activity. These data suggest cortical NE is not critical for acquisition or maintenance of CAR in F rats, but hint at a possible role in S/D rats. Further work may be required to determine whether cortical NE mediates a segment of CAR behavior in S/D rats. In this regard, Ogren et al (Neurosci: Let. 1980,20:351) have shown DSP-4-induced decrements in acquisition of a passive 1 or 2 way avoidance task in S/D rats.

348.5 DEPRESSION OF LOCOMOTOR BEHAVIOR OF MICE BY NORCOCAINE. M.E.A. Reith* and A. Lajtha. Center for Neurochemistry, N.S. Kline Institute for Psychiatric Research, Ward's Island, New York, NY 10035.

It has been found in a number of laboratories that cocaine produces an increase in spontaneous locomotor behavior of mice and rats. Recent studies in our lab on structure-activity relationships of this effect of cocaine showed that most cocaine congeners inhibit rather than stimulate spontaneous locomotion of young adult male BALB/cBy mice. Locomotor depression was observed after IP administration of norcocaine, (+)-pseudococaine, tropacocaine, or phenyltropane analogs of the WIN-series. Hypomotive effects have been reported also for $\alpha_2$-adrenergic agonists such as clonidine, presumably by interactions with central $\alpha_2$-adrenoceptors.

In the present study we tested whether central $\alpha_2$-adrenoceptors are involved in the locomotor depression produced by cocaine congeners, since cocaine has been suggested to interact with the imidazoline recognition site of the $\alpha_2$-adrenoceptor in the CNS. Spontaneous locomotion was measured by placing individually housed mice in their home cages in an Opto-Varimex-Minor activity monitor (Columbus Instruments) equipped with infrared beams. The behavioral data were based on interruptions of consecutive beams only. Each animal was monitored for 40 min to establish his baseline. The animal was then injected with an antagonist or saline and monitored for 20 min. Subsequently he received an injection of agonist, norcocaine, or saline, and was monitored for another 30 min. The inhibition of locomotion by norcocaine (10 mg/kg IP) was not antagonized by the adrenoceptor antagonists yohimbine (2 mg/kg SC) and phentolamine (0.3 mg/kg IP). In contrast, these doses were effective in reducing the hypomotility induced by clonidine (0.1 mg/kg IP). It is therefore unlikely that central $\alpha_2$-adrenoceptors are involved in the hypomotive effect of norcocaine. Since presynaptic dopamine receptors have been implicated also in hypomotive effects of drugs, we studied the effect of haloperidol (0.1 mg/kg IP) and spiperone (0.05 mg/kg IP) on the norcocaine-induced inhibition. These low doses aimed at presynaptic dopamine receptors did not decrease the locomotor inhibition. The data so far are consonant with the view that the locomotor inhibition observed with IP norcocaine and other cocaine congeners is due to the local anesthetic potency of cocaine-related structures, and is not mediated by either central $\alpha_2$-adrenoceptors or presynaptic dopamine receptors.

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348.6 EFFECTS OF CENTRALLY ADMINISTERED HISTAMINERGIC AGENTS IN THE BEHAVIORAL DESPAIR TEST. K.A. O'Neill and S.B. Gertner*. Dept. of Pharmacology, UMDNJ - New Jersey Med. Sch. Newark, NJ 07103

Antidepressant drugs (AD), electroconvulsive shock and REM sleep deprivation significantly increase the swimming time of mice and rats in the behavioral despair test. Other agents which have shown similar activity include the classical $H_1$ antagonists. These drugs are of particular interest because both tricyclic and atypical antidepressants potently block mepyramine binding at the $H_1$ receptor (Hall & Ogren, Eur.J.Pharmac.,70:393,1981). In addition, $H_2$ receptor mediated stimulation of hippocampal adenylate cyclase is also blocked by AD (Green & Maayani, Nature,269:163, 1977). These observations suggest that central histaminergic systems may be involved in depression.

To explore the behavioral significance of these effects, experiments were conducted to determine the actions of specific histamine agonists and antagonists in the behavioral despair test. CD-1 male mice received intracerebroventricular (ICV) injections of drugs or vehicle (distilled $H_2O$ or artificial cerebrospinal fluid) into the lateral ventricles, in a constant volume of 5 ul. Mice were placed in beakers of 25°C water 2-15 min after injections, for 6 min, and swimming behavior was rated and ranked for the final 4 min. Data shown are ranks, and were analyzed by the Mann-Whitney U test.

Control mice tested at different times after injections did not show significantly different swimming behavior. Therefore, control group data were combined yielding a mean rank of 5.3. The $H_2$ antagonist, cimetidine (10,25 & 50 ug) caused a dose-related reduction in swimming (4.0,2.4, & 2.5), as did the non-imizadole $H_2$ antagonist, BMY 25,368 (10,20 & 25 ug; 3.4,2.5 &2.0), 15 min after administration. This effect was attenuated by the $H_2$ agonist, impromidine (25 & 50 ug), and surprisingly, by the $H_1$ antagonist, chlorpheniramine (50 & 100 ug). Histamine (HA) (1,2.5,5 & 10 ug) had no effect on swimming 2 min after injection (5.4,3.8,3.6 & 4.0). HA (5 ug) given with the HA methyl transferase inhibitor SKF 91488 slightly reduced swimming (3.0), and the two compounds together had no effect on the actions of the $H_2$ antagonists. The specific $H_1$ agonist, pyridylethylamine (50 & 100 ug), and the $H_2$ agonist, impromidine (25 & 50 ug) were also without effect at 2, 5 & 10 min. Although others have shown that $H_1$ antagonists given peripherally are active, chlorpheniramine at 50 or 100 ug ICV had no effect on swimming.

These results suggest that $H_1$ and $H_2$ receptors may mediate opposing actions within the CNS. $H_2$ receptor blockade induced a depressive like state, which was attenuated by a specific $H_2$ agonist, as well as an $H_1$ antagonist. Taken together, these data suggest that disturbances of CNS histaminergic systems may play a role in depression.

348.7 HISTAMINERGIC (H1) INVOLVEMENT IN VISCERAL LEARNING. P. S. Lasiter, M. D. Holder* and J. Garcia*. Dept. of Psychology and Brain Research Inst., Univ. of Calif., Los Angeles, CA 90024.

Histamine receptors, neurons and terminal endings are distributed within brain regions that contribute to special visceral functions. These regions include the amygdala, hypothalamus, dorsolateral parabrachial region, and the caudal solitary nucleus (e.g., Palacios, J.M., et al., Neurosci., 6: 15, 1981; Watanabe, T., et al., Brain Res., 295: 13, 1984). Our experiments examined the involvement of histaminergic systems in one form of visceral learning, namely, conditioned taste aversion (CTA) learning. In this conditioning procedure, taste consumption is followed by illness; Rats subsequently exhibit robust aversions to a taste that is consumed prior to illness. In Experiment 1, distinct groups of male hooded rats received intraperitoneal injections of either pyrilamine maleate (10, 15, 20 or 30 mg/kg), triprolidine HCl (10, 20 or 25 mg/kg), chlorpheniramine maleate (5, 10 or 15 mg/kg) [histamine H1 ligands], or cimetidine (50 or 100 mg/kg) [histamine H2 ligand]. Control animals in each group received vehicle injections only. Forty-five minutes following injections one-half of the experimental and the control animals drank 100 mM LiCl to induce illness and the remaining animals drank 100 mM NaCl to assess normal taste preferences to a "salty" taste (Lasiter, P.S., Behav. Neural Bio., 39: 149, 1983). Two days following conditioning all animals received 100 mM NaCl or distilled water on an alternating daily schedule to evaluate CTAs. Results showed that H1 and H2 ligands did not obviously alter normal taste preferences to 100 mM NaCl nor gastrointestinal reactivity to 100 mM LiCl, yet CTA learning was impaired in a dose-dependent manner only in animals receiving H1 ligands. Experiment 2 determined if injections of histamine ligands produced gastrointestinal disturbances in Experiment 1. Rats drank 100 mM NaCl prior to injections of pyrilamine (20 mg/kg), triprolidine (20 mg/kg), cimetidine (100 mg/kg), or LiCl (67 mg/kg). Injections of histamine ligands following taste consumption did not produce CTAs, indicating that the ligands did not produce illness at the specific doses used in Experiment 1. In Experiment 3 rats received injections of either alpha-hydrazino histidine (a-HH), a histamine synthesis inhibitor, (3 injections; 100 mg base/kg/12 hr), l-histidine HCl (3 injections; 100 mg base/kg/12 hr), or saline prior to CTA conditioning. Results showed that only a-HH injections impaired CTA learning. Thus, attenuation of histamine synthesis yielded behavioral effects similar to those obtained with H1 ligands. These results indicate that H1 histaminergic systems can be classified as at least one substrate for visceral learning functions. Although "antihistamines" have been classified as antiemetic agents, our results suggest that H1 ligands may affect the higher-order interpretation of illness, rather than mechanisms of the emetic response per se. (Supported by NIHNCDS R01-11618; NIH HD- 050958)

## BEHAVIORAL PHARMACOLOGY: NEUROLEPTICS AND DOPAMINE

349.1 SOME EFFECTS OF PIMOZIDE ON NONDEPRIVED RATS LEVER PRESSING FOR A SUCROSE SOLUTION IN AN ANHEDONIA PARADIGM. S.E. Gramling*, S.C. Fowler, and J.P. Tizzano*. Dept. of Psych., Univ. of Miss., University, MS 38677.

The apparent similarities in patterns of responding occasioned by either neuroleptic treatment or by extinction procedures in operant appetitive tasks is often cited to support the "anhedonia" (reward-reducing) interpretation of neuroleptics rate reducing effects. A recent study (Gramling, S.E., Fowler, S.C., & Collins, K.R., Pharmacol Biochem Behav, 21, 617-624, 1984) which tested rats in a "traditional" anhedonia paradigm reported that nondeprived rats trained to lick a 32% sucrose solution did not produce extinction-like patterns of responding across eight consecutive days of treatment with pimozide (PIM). The present experiment extended this research by testing nondeprived rats in a similar procedure but employing a leverpress response.

Thirty nondeprived rats leverpressed at stable rates for a 32% sucrose solution reward on a CRF schedule and were then randomly assigned to one of five treatment conditions. Rats in the reward (RWD) condition received vehicle injections and responded for 32% sucrose solution in the test situation. Rats in an extinction (EXT) condition received vehicle injections but no sucrose. Two additional groups received injections of PIM (0.25 mg/kg or 0.5 mg/kg) and responded for normal reward in the test situation. All rats received their respective treatments for eight consecutive days and all injections preceded data collection by four hours. Response rate and duration data were collected via a laboratory computer system.

Rats in the EXT condition exhibited a monotonic decrease in rate across the eight test days. Rats in both drug groups, however, exhibited a significant curvilinear pattern wherein initial rate decreases were followed by a trend towards recovery across the final three days of testing. The duration data also revealed differences between PIM treatment and extinction procedures in that PIM treatment did not lengthen response duration to the same extent as extinction procedures.

Both the present experiment and the Gramling et al. (1984) study observed across-session curvilinear patterns of responding in PIM treated rats. These two experiments differed from other tests of the anhedonia hypothesis in their use of nondeprived rats, a natural reinforcer of high hedonic value, and an extended dosing regime. The failure to obtain extinction-like patterns of responding with these procedures suggests that anhedonia may not be an adequate explanation of PIM's extinction-like effects observed in other settings.

349.2 THE EFFECTS OF PIMOZIDE ON STIMULUS DISCRIMINATION AND REWARDING ELECTRICAL BRAIN STIMULATION. M. Bird* and C. Kornetsky. Lab. Behav. Pharmacol., Boston Univ. Sch. Med., Boston, MA 02118

Pimozide, a selective dopaminergic antagonist, has been shown to cause a dose dependent inhibition of intracranial self-stimulation (Wise, R.A. Pharmacol. Biochem. Behav. 13:213-223, 1980). This phenomenon has often been interpreted as the result of selective pharmacologic blockade of the rewarding quality of brain stimulation and not a function of generalized disruption of performance capabilities.

In an attempt to more conclusively address these issues, intracranial rewarding stimulation and detection thresholds were measured after intraperitoneal injection of pimozide. The threshold level of stimuli employed in the detection procedure were neither positively or negatively reinforcing and were used solely as a discriminative cue.

Bipolar stainless steel electrodes aimed at the medial forebrain bundle - lateral hypothalamic area (MFB-LH) were stereotaxically implanted in male albino rats (CDF - Charles River Laboratories). Following surgery the animals were trained on a rate-free method of determining the threshold for rewarding brain stimulation (Esposito, R.U. and Kornetsky, C. Science, 195:189-191, 1977) and the effects of pimozide intraperitoneally administered on this threshold were determined. The same animals then were trained on a method for determining the threshold for detecting non-rewarding intracranial stimulation. The procedure required the animal to make an instrumental response to a 0.5 sec, low intensity, non-rewarding cue (S1) to the same brain site used in the reward procedure (Wheeling, H.S. and Kornetsky, C. Brain Res., 272:13-19, 1983). Responding to the cue within 5 sec was maintained by the delivery of a reinforcing stimulus (S2) via the same electrode. Absolute detection threshold were determined by varying the current intensity of the brain stimulation cue (S1) according to a modification of the psychophysical method of constant stimuli. The reinforcing stimulus (S2) remained at a fixed intensity level clearly above the reward threshold previously determined after moderate doses of pimozide.

Preliminary results indicate that moderate doses of pimozide (0.2 mg/kg) cause an increase in the threshold for both reward and detection brain stimulation. However, the ability of the animals to discriminate between the intensity of stimulation was more impaired in the detection than the reward paradigm. The differential effects of pimozide on the reward and detection threshold profiles suggest that pimozide not only decreases the rewarding value of reinforcing brain stimulation but also impairs the perception and discrimination processing of non-rewarding stimuli. (Supported in part by NIDA grant DA 02326 and NIDA Research Scientist Award (CK) K05 DA 00099).

349.3 COCAINE SELF-ADMINISTRATION AND HORMONAL STATUS: THE ESTROUS CYCLE AND THE ANTIESTROGEN TAMOXIFEN. J.C.H. Dalton*, G.J. Vickers* and D.C.S. Roberts. Dept. of Psychology, Carleton Univ., Ottawa, Canada, K1S 5B6.

Rats pretreated with small doses of antipsychotic drugs show an increase in cocaine self-administration, and this increase has been shown to strongly correlate with clinical dosage (Roberts & Vickers, Psychopharm. 82:135, 1984). We have suggested that the mechanism which causes this increase in cocaine intake may be distinct from extrapyramidal effects, and therefore this procedure may provide a useful model for investigating the therapeutic effects of neuroleptic drugs. Both clinical and basic research now indicates that female sex hormones can affect the response to dopamine antagonists. We have employed the cocaine self-administration procedure to characterize the role of female hormonal status on the "therapeutic potency" of neuroleptic drugs.

We have previously shown that ovariectomy (OVX) attenuates haloperidol-induced increases in cocaine self-administration but does not affect baseline intake as compared to control animals (Neurosci. Abstr. #245.11, 1984). We now report that the potency of haloperidol fluctuates across the estrous cycle. Female Wistar rats were implanted with chronic jugular cannulae and permitted to self-administer cocaine (1.5 mg/kg/inj for 4 hrs/day). The effect of haloperidol pretreatment (0.1 mg/kg, one hr prior to testing) was examined in animals which showed a stable intake of cocaine, and displayed a regular estrous cycle. Haloperidol was found to produce a significantly greater increase in cocaine intake during diestrous (97%) compared to animals tested during either proestrous (75%) or metestrous (78%).

In a separate experiment, it was shown that the increase in cocaine self-administration normally produced in female animals (counter-balanced across the estrous cycle) was attenuated by a single injection of the antiestrogen tamoxifen (1.0 mg/kg, 24 hrs prior to testing).

These data demonstrate that normal physiological fluctuations in hormonal balance or pharmacological inhibition of estrogen function can affect the response to neuroleptic drugs, and indicate that hormonal status may be a significant influence on neuroleptic efficacy. (Supported by the Medical Research Council).

349.4 EFFECTS OF THE FATTY ACID CONJUGATE PALMITOYLCODEINE ON BEHAVIORAL AND NEUROCHEMICAL MEASURES IN THE RAT. G.C. Haggerty*, R. Deskin, A.F. Fentiman* and E.Leighty*. Battelle Columbus Laboratories, Columbus, OH 43201.

Studies have shown that palmitic acid can be conjugated with codeine in an in vitro coenzyme A fortified rat microsomal incubation system to form palmitoylcodeine (Leighty et al, J. Pharm. Pharmacol. 35: 260-261, 1983). The objective of the present studies was to determine if palmitoylcodeine is pharmacologically active in the rat. For comparison purposes, a codeine dosed group was included as a positive control. Both codeine and palmitoylcodeine (2 or 4 times the equimolar dose of codeine) decreased thermal sensitivity and spontaneous motor activity at 1 hr, and induced a state of catalepsy up to 2 hr in rats following intravenous administration. Palmitoylcodeine-induced changes in thermal sensitivity and motor activity followed a temporal pattern of response similiar to that seen with codeine, but the effects were less pronounced. While the catalepsy produced by codeine was seen almost immediately after injection, the palmitoylcodeine-induced response developed more slowly. Although neither compound exerted an effect on hypothalamic norepinephrine (NE) uptake at 2 hr post administration, striatal dopamine (DA) uptake was significantly ($P<0.05$) decreased in the codeine dosed group and tended also to be depressed in palmitoylcodeine-treated animals. These effects on DA uptake are of interest since dopaminergic mechanisms are believed to play a role in the development of both catalepsy and locomotor activity. To further characterize the observed palmitoylcodeine-induced behavioral changes, animals were intracisternally injected with codeine or the fatty acid conjugate. In contrast to the sedation observed after intravenous administration of these compounds, animals from both the codeine and palmitoylcodeine groups were agitated and exhibited an increase in spontaneous motor activity. The results of these studies demonstrate that palmitoylcodeine is psychoactive. The fatty acid conjugate produced effects similar to those of codeine, and, for both compounds, the route of administration appeared to be an important determinant of the type of behavioral effects induced. The observed behavioral responses may be due to the pharmacological activity of palmitoylcodeine itself and/or its metabolic conversion to psychoactive codeine (supported by NIDA Grant No. DA00793-07).

349.5 A COMPARISON OF THE EFFECTS OF APOMORPHINE, (+)- and (-)-3-PPP AND HALOPERIDOL ON THE FIGHTING BEHAVIOR AND MOTOR ACTIVITY OF DIFFERENTIALLY HOUSED MICE. T. Fico, C. Wilmot, and C. VanderWende. Rutgers Univ., Dept. Pharmacology, PO Box 789, Piscataway, NJ 08854

The individual housing of male mice is noted for producing a prominent fighting behavior, a heightened reactivity and an increased monoamine turnover in response to a novel environment. The present study was conducted to determine (1) if the fighting behavior could be modulated by low doses of the DA agonists, apomorphine (APO) and (+)-3-PPP, the mixed agonist (-)-3-PPP and the DA antagonist haloperidol (HAL), (2) the relationship between the effects of these agents on motor activity and fighting behavior and (3) to compare the motor activity response of group housed (GH) and individually housed (IH) mice. Male CF-1 mice were housed either individually or in groups, 15/cage, for 4 wks, starting at 34-38 days of age. IH mice were tested for fighting behavior with an olfactory bulbectomized stimulus mouse, which does not initiate a fight nor retaliate. Motor activity was measured in separate groups of age-matched GH and IH mice.

APO, 0.01875-0.3 mg/kg, and (+)-3-PPP, 0.7-4.0 mg/kg, significantly reduced the activity of IH mice, whereas fighting behavior was affected only by higher doses, 0.3 - 0.6 mg/kg APO and 4.0-12.0 mg/kg (+)-3-PPP. In contrast, (+)-3-PPP, 0.7-4.0 mg/kg, reduced both the number of mice fighting and motor activity. HAL, 0.5 mg/kg, inhibited fighting, however, 0.125-0.5 mg/kg HAL reduced motor activity. IH mice were more sensitive than GH mice to the activity-reducing effects of APO and both enantiomers of 3-PPP. These results indicate that low doses of DA agonists selectively reduce motor activity and only higher doses reduce fighting behavior. Also, individual housing produces an increased sensitivity to the hypomotility response to DA agonists.

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349.6 EFFECTS OF NUCLEUS ACCUMBENS 6-HYDROXYDOPAMINE LESIONS ON RESPONDING CONCURRENTLY MAINTAINED BY FOOD, WATER AND MORPHINE. J.E. Smith, K. Schultz* and S.I. Dworkin. Psychiatry Research Unit, Departments of Psychiatry and Pharmacology, Louisiana State University Medical Center, Shreveport, LA 71130.

The neurotoxin, 6-hydroxydopamine (6-OHDA), has been used to investigate neurobiological aspects of reinforcement. 6-OHDA can be used to selectively destroy dopamine containing neurons in discrete brain regions. Such lesions of the nucleus accumbens affect food, water and drug self-administration. 6-OHDA lesions of this structure decrease or eliminate cocaine and amphetamine self-administration and either increase or do not affect opiate self-administration in rats with unrestricted access to food and water. However, there have been very few investigations of the effects of neurotoxin lesions on responding simultaneously maintained by several different reinforcers. This study reports the effects of 6-OHDA lesions of the nucleus accumbens on responding concurrently maintained by food, water and morphine.

Eight male rats were trained on a concurrent fixed-ratio 10 schedule of reinforcer presentation with continuous access. Three response levers were available to the subject. A single response on any lever resulted in the retraction of the other two. Nine additional responses resulted in the delivery of either a 45 mg food pellet, 0.1 ml of water or 10.0 mg/kg infusion of morphine, depending on the lever selected. After stable patterns of responding maintained by the three reinforcers were obtained, dose-effect curves for morphine were obtained. Other doses of morphine (2.5-40 mg/kg) were substituted for the 10.0 mg/kg dose for 24-hour periods. The effects of both sham vehicle and 6-OHDA (4 ug in 0.5 ul delivered over 7 minutes) lesions were then determined and the dose-effect evaluations repeated.

6-OHDA lesions did not significantly affect responding maintained by food, water or morphine. This is in contrast to previous reports of the neurotoxin on drug self-administraton procedures. The absence of an effect is most likely not the result of an insensitive baseline. Kainic acid lesions produce a selective decrement in morphine self-administration using this procedure.

Although these data appear contradictory to those obtained using one reinforcer, they may not be. This lesion may initially severely attenuate responding maintained by a variety of reinforcers. However, in this procedure, a decrement in food and water intake could have fatal consequences. Therefore, previously redundant secondary systems may be elaborated. (Supported in part by USPHS Grant DA 01999-08).

349.7 SEX DIFFERENCES IN NEUROLEPTIC-INDUCED CATALEPSY. M.H. Lewis, M.F. Keresztury*, R.B. Mailman. Department of Psychiatry, University of Medicine and Dentistry of New Jersey - School of Osteopathic Medicine, Camden, NJ 08103 and Biological Sciences Research Center, University of North Carolina, Chapel Hill, NC 27514.

Gonadal steroids, particularly estrogen, have been reported to modulate dopamine neurotransmission, although data on the nature of such modulation are contradictory.

The present study examined sex differences in the cataleptogenic potency of three structurally dissimilar dopamine antagonists: thioridazine, haloperidol and SCH23390. Following intraperitoneal (ip) drug administration, intact male and female Sprague-Dawley rats were tested by placing the animal's forepaws on a 10 cm high metal bar. Dose-response relationships were evaluated by measuring the latency to placement of both forepaws on the table surface at 15 minute intervals following drug treatment.

Thioridazine was found to be weakly cataleptogenic although significantly more potent in males ($ED_{50}$=ca. 85 mg/kg) than females. To test whether this difference may have been due to differential drug metabolism, blood and brain concentrations of thioridazine and its active metabolites were quantified in male and female rats by HPLC. Also, catalepsy was assessed following administration of mesoridazine, an active S-oxidized metabolite of thioridazine. Mesoridazine was approximately twice as potent as thioridazine in inducing catalepsy yet equipotent in males and females. Additionally, thioridazine proved to be equipotent in inducing catalepsy in male versus ovariectomized female rats.

Haloperidol was found to be significantly less potent in male than in female ($ED_{50}$=ca. 0.5 mg/kg) rats. In order to determine if the observed difference was due to the effect of gonadal steroids on drug metabolism, haloperidol was injected intracerebroventricularly (icv) into intact males and females. The sex difference previously observed after ip injection was no longer apparent following icv administration.

No significant sex differences in cataleptogenesis were observed following ip administration of the purportedly selective D1 antagonist SCH23390 ($ED_{50}$=ca. 0.1 mg/kg). Drug concentrations in blood and brain of male and female rats as determined by HPLC complemented the behavioral results observed.

It appears that gonadal steroids may alter substantially the bioavailability of at least some dopaminergic drugs. Such action may explain, in part, the contradictory data on the modulatory role of gonadal steroids on dopamine function.

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349.8 HALOPERIDOL-INDUCED DEFECATION IN LABORATORY RATS: PERIPHERAL OR EMOTIONAL EFFECT? S.H. Hagenmeyer, K.H. Russell,* M.D. Bunsey* and P.R. Sanberg, Behavioral Neuroscience Laboratory, Dept. of Psychology, Ohio University, Athens, OH 45701.

Given under certain conditions, the dopamine (DA) receptor blocker haloperidol (HAL) produces increased defecation, as measured by the number or weight of fecal boli in laboratory rats. Although defecation is used an an index of emotionality in animals, the fact that a major tranquilizer can increase defecation suggests the need for an examination of the underlying mechanisms for this drug-induced phenomenon. The present study examined whether the HAL effect was related to peripheral DA receptors or emotionality by using the peripheral DA receptor blocker, domperidone, and the antianxiety agent, diazepam, respectively.

Male Sprague Dawley rats (about 370 gm) were injected i.p. in their homecage with either 0.1 or 1.0 mg/kg of HAL or domperidone. Fecal boli counts were taken hourly and massed at the end of a three hour test session. Other studies were performed on the effects of HAL on day vs night and homecage vs openfield defecation. Finally, defecation was studied in animals pre-injected i.p. with diazepam (0.5, 1.0, or 2.0 mg/kg) and given HAL (1.0 mg/kg) or vehicle one hour later.

The results indicated an overall increase in defecation measures with HAL but not domperidone. Nocturnal fecal excretions were consistently greater than those during the daytime. However, the nocturnal animals defecated the most during the third hour post-injection (22:00-23:00) in comparison to the daytime where the first hour showed the largest defecation rates. This may be related to the fact that one of the periods of greatest locomotor activity falls between 22:00 and 23:00. Following a five hour habituation period, the open-field vs homecage defecation study demonstrated significantly more defecation in the homecage. Finally, rats injected with diazepam and HAL defecated significantly less than those animals which received HAL alone. There were no differences between the diazepam/HAL group and the diazepam/vehicle group.

These findings reveal that a peripheral DA receptor blocker does not significantly increase defecation behavior in rats. This suggests that the underlying mechanism for HAL-induced defecation is not an effect on peripheral DA receptors located in the gastrointestinal tract. On the other hand the differences in homecage vs open field defecation suggest that the neuroleptic may be enhancing emotionality in the rat. This is further supported by the results that those animals which received both diazepam and HAL had normal fecal excretions. It is suggested that the involuntary restruction of locomotor mechanisms in the animal by HAL may produce high levels of anxiety, thereby increasing defecation.

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349.9 CHRONIC, BUT NOT ACUTE, ESTRADIOL TREATMENT ALTERS THE APOMORPHINE "CUE". J. T. Concannon and M. D. Schechter. Program in Pharmacology Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272.

Adult male rats were trained to discriminate the interoceptive cue produced by intraperitoneally administered apomorphine (APO) in a 2-lever, food-motivated operant task. After reaching a criterion of 90% drug-appropriate responses at the training dose of 0.16mg/Kg, animals were injected subcutaneously with 100µg/Kg/day estradiol benzoate for 3 consecutive days. The first injection was administered 1 hr prior to the apomorphine training dose, whereas the second dose was administered 1 hr prior to saline. The results of first two injections showed that "acute" estradiol administration did not affect the cue produced by either the training dose of apomorphine or saline. The experiment was continued by administering a third dose of estradiol the next day, and then testing the response to a previously-determined ED50 (0.08mg/Kg) dose of apomorphine 1, 2, 4, and 8 days after estradiol "withdrawal".

The results of the chronic experiment are shown in the table:

| Post-estradiol day | Dose | %APO Lever Selection |
|---|---|---|
| 1 | 0.08 APO | 60.0 |
| 2 | 0.08 APO | 100.0 |
| 4 | 0.08 APO | 60.0 |
| 8 | 0.08 APO | 60.0 |

It was thus observed that the 3 consecutive estradiol injections augmented the animals' response to the 0.08mg/Kg APO dose, i.e., there was behavioral supersensitivity. Furthermore, this supersensitivity was time-dependent, emerging 2 days after the last estradiol injection, and disappearing just 2 days later. These results are formally similar to those produced by chronic antipsychotic (anti-dopaminergic) drug administration, which also produces behavioral supersensitivity to dopamine agonists in a time-dependent manner. This similarity leads to the suggestion that estradiol may be anti-dopaminergic. Further research is needed, however, to determine the underlying neuropharmacological mechanism of estradiol's action in order to test the validity of this inference.

349.10 STRENGTHENING OF ONGOING ACTIVITY BY APOMORPHINE: EFFECT ON SEXUAL BEHAVIOR. Henry Szechtman. Dept. of Neurosciences, McMaster Univ., Hamilton, Ont., CANADA, L8N 3Z5.

Dopaminomimetic drugs have been shown to induce stereotyped behavior that reflects "the behavioral pattern which was ongoing when the first infusions of drug were received" [1]. Also, self-administration studies have shown that these drugs have reinforcing effects [2]. These findings have been taken to support the hypothesis that dopaminomimetic drugs reinforce "the behavior displayed during the onset of drug action" [3]. This study tests the generality of this hypothesis by determining whether the dopamine agonist, apomorphine, strengthens ongoing sexual behavior. Contrary to the hypothesis, the findings indicate that the drug diverts the rats' attention from sexual activity to performance of behaviors typically exhibited by rats injected with apomorphine.

Twenty-six male Sprague-Dawley rats received extensive sexual experience (10 weekly 20 min sessions). They were administered apomorphine (1.25 mg/kg s.c.), or saline, on mating sessions #11 and #12. Half the animals received the drug first, and the other half, saline first. Injections were given after the males achieved the second intromission; testing ended 20 min later.

All males ejaculated at least once after injection of saline (mean ± sem number of ejaculations: 2.0 ± 0.2). However, after injection of apomorphine, 17 rats did not continue to copulate until ejaculation; the remaining 9 rats ejaculated only once. All animals showed behavior typical of apomorphine-treated rats, ie., snout contact fixation, forward progression, and turning [4].

These results indicate that there is a limit to the kind of ongoing behavior that can be reinforced by apomorphine. Furthermore, the fact that males ceased to copulate, an activity that is not interrupted by the availability of food in severely starved rats [5], suggests that apomorphine is extremely potent in redirecting the animals' responsiveness to some unique range of stimuli. (Supported by MRC. HS is a MRC Scholar.)

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349.11 **THE EFFECT OF DOPAMINE AUTORECEPTOR AGONISTS ON EXPLORATORY AND DRUG-STIMULATED LOCOMOTOR ACTIVITY IN RATS.** P.J.K.D.Schreur and N.F.Nichols*, CNS Research, The Upjohn Company, Kalamazoo, MI 49001.

In several areas of the brain, dopamine secreted from neuron terminals acts on "autoreceptors" on the same terminals to decrease their further release of dopamine. Thus, transmission at dopaminergic synapses can be inhibited by administration of either a dopamine autoreceptor agonist or a postsynaptic antagonist (e.g., a neuroleptic).

**Methods.** The locomotor activity of male Sprague-Dawley rats was measured automatically in Omnitech Digiscan activity monitors. Drug-stimulated behavior (d-amphetamine $SO_4$ 0.3 mg/kg or apomorphine HCl 0.5 mg/kg s.c.) was tested for 60 min after drug injections, in rats which had been habituated to the cages for 40 min in the light (n = 8-11). Exploratory behavior was tested for 10 min in the dark, in naive rats which had been preinjected with drug in the home cage (n = 8).

**Results, Exploratory Activity.** The potential autoreceptor agonists apomorphine HCl, NPA (N-n-propylnorapomorphine), and PPP (N-n-propyl-3-(3-hydroxyphenyl)-piperidine) and its enantiomers decreased the total distance travelled by exploring rats while at the same time paradoxically increasing the number of discrete movements. This is a very different pattern from that of the typical antipsychotic drugs haloperidol and chlorpromazine, and the atypical antipsychotic drug clozapine, which also decreased the total distance travelled but decreased the number of movements. Both groups decreased the distance/movement.

**Results, Drug-stimulated Activity.** Apomorphine (0.001-0.1 mg/kg), NPA (0.001 mg/kg), and (+)-PPP (1 mg/kg) antagonized amphetamine-stimulated locomotor behavior (total distance) without antagonizing apomorphine-stimulated behavior, suggesting a presynaptic dopamine autoreceptor agonism. On the other hand, haloperidol, chlorpromazine, and clozapine decreased both amphetamine- and apomorphine-stimulated behavior, suggesting a postsynaptic dopamine antagonism. PPP and (-)-PPP showed neither pattern in this test.

349.12 **DISCRIMINATION OF A COMPOUND DRUG STIMULUS: AMPHETAMINE PLUS LSD VERSUS SALINE.** J. M. Hanlin* and J. B. Appel. Behavioral Pharmacology Laboratory, Department of Psychology, University of South Carolina, Columbia, SC 29208.

To test the hypothesis that animals can discriminate pharmacologically distinct components of drug-induced cues, rats (N=12) were trained to discriminate a combination of (+)-amphetamine (1.0 mg/kg, i.p.) and LSD (0.08 mg/kg, i.p.) from saline. A two-lever procedure involving an FR 10 schedule of water reinforcement was used.

The discrimination was learned rapidly and was maintained at a high level of accuracy. In subsequent substitution (generalization) and antagonism tests, the training doses of amphetamine and LSD consistently elicited about 80% and about 20% drug-lever responding, respectively; the dopamine (DA) antagonist haloperidol blocked the effect of amphetamine but increased that of LSD, whereas the serotonin-2 (5HT-2) antagonist ketanserin blocked the effect of LSD but did not alter that of amphetamine. The amount of antagonism of the amphetamine-LSD combination by ketanserin was proportional to the amount of generalization of the combination to the training dose of LSD (about 20%). Haloperidol partially antagonized the combination (to 54.0%) at a dose of 0.5 mg/kg; higher doses were less effective. These results support the hypothesis that components of drug cues can be discriminated.

Results of tests with lower and higher doses of the two training drugs suggest that the LSD-like portion of the compound cue may be larger than the serotonergic portion, i.e., that amphetamine may contribute to the LSD-like component through a non-serotonergic mechanism. Generalization of the training combination to LSD occurred with a steep slope and peaked at 74.5% at a dose of 0.32 mg/kg; generalization to amphetamine also occurred with a steep slope and reached 99.2% at 1.5 mg/kg. In rats trained with (+)-amphetamine (1.0 mg/kg) alone, generalization to LSD occurred with a shallow slope and peaked at 38.6% at a dose of 0.24 mg/kg. Such results suggest that amphetamine (given subchronically) may have two discriminable effects, a DA-mediated central stimulant effect that is of relatively high salience (is more discriminable) and a DA-mediated LSD-like effect that is of relatively low salience (is less discriminable); addition of LSD to the amphetamine training regimen appears to increase the salience of the LSD-like component of the amphetamine cue.

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## NEUROTOXICITY IV

350.1 PRENATAL CARBON MONOXIDE EXPOSURE CAUSING LOW LEVEL HYPOXIA LEADS TO A SIGNIFICANT INCREASE IN STRIATAL DOPAMINE (DA), A DECREASE IN DA SYNTHESIS, BUT NO CHANGE IN DA METABOLITE LEVELS. M.J. Kaufman, M.B. Upchurch*, and L.D. Fechter. Neurotoxicology Program, Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD 21205.

The developing central nervous system seems to be quite sensitive to hypoxia, as shown by behavioral changes and learning and memory deficits (Mactutus and Fechter, 1984; Science 223:409-411). The striatum in particular appears vulnerable to hypoxia, especially to that induced by carbon monoxide (CO) exposure. Further, tyrosine hydroxylase, the rate limiting enzyme in catecholamine biosynthesis, is oxygen requiring and might be affected if oxygen tension is reduced. The present experiment was designed to study the effects of CO-induced hypoxia on catecholamine and metabolite levels in the developing striatum.

Pregnant Long-Evans hooded rats were exposed from day 1 of gestation to postnatal day 10 with their litters to either 0, 75, 150, or 300 ppm CO. Striata from pups sacrificed at postnatal ages 10, 14, or 21 days were collected for subsequent assay of catecholamines and metabolites by HPLC-EC. Additional 0 or 300 ppm CO-exposed, 21-day-old rats were pretreated with m-hydroxybenzylhydrazine to inhibit dopa decarboxylase activity 0, 10, 20, or 30 minutes before sacrifice. Accumulation of dopa was measured in these animals as an estimate of the rate of DA synthesis.

DA levels were significantly increased in a dose-response fashion in 21-day-old rats (one way ANOVA: $F(3,28) = 3.12$, $p < 0.05$). In addition, a two way ANOVA indicated that CO exposures increased DA levels across all ages of sacrifice ($F(3,71) = 4.149$, $p < 0.01$). The rate of DA synthesis, however, was significantly reduced in 300 ppm exposed animals. DA metabolite levels were not increased, suggesting the possibility that excess DA is stored and unused. Since these elevations in DA levels and reductions in DA synthesis rates are seen at postnatal day 21, long after termination of CO exposure, they are indications of persisting damage sustained by the nigrostriatal dopaminergic projection system.

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350.2 A-METHYLPARATYROSINE (AMT) PREVENTS THE METHYLAMPHETAMINE (MA)-INDUCED FORMATION OF ENDOGENOUS 5,6-DIHYDROXYTRYPTAMINE (5,6DHT) IN RAT HIPPOCAMPUS. K.J. Axt*, D.L. Commins*, L.S. Seiden. (SPON: S.P. Grossman), Department of Pharmacol. & Physiol. Sci., University of Chicago, Chicago, IL 60637.

We now report that a single high dose of MA (100 mg/kg s.c.) produces a significant depletion of serotonin (5HT) in the hippocampus of the male Sprague-Dawley rat. These 5HT depletions average across experiments to 50% of control values when measured 2 weeks after drug administration. In addition, we have found that acute MA administration causes the endogenous formation of 5,6DHT which is blocked by AMT.

Prior administration of the catecholamine synthesis inhibitor AMT (150 mg/kg s.c., 6 and 1 hr prior to MA) prevents MA-induced long-term hippocampal 5HT depletions. 5HT levels in AMT+MA rats are 94% of those of the AMT+saline controls whereas 5HT levels of the saline+MA rats are 25% of those of the saline+saline controls. However, pretreatment with a dose of the 5HT synthesis inhibitor parachlorophenylalanine (PCPA, 300 mg/kg i.p., 48 and 24 hrs prior to MA) which reduces hippocampal 5HT levels to less than 1% of control values at the time of MA injection, fails to block the MA-induced 5HT depletion. 5HT levels in PCPA+MA rats are 69% of those in PCPA+saline controls, compared to 5HT levels in vehicle+MA rats which are 65% of those in the vehicle+saline controls. In light of our recent observation that the 5HT neurotoxin 5,6DHT is formed endogenously in rat hippocampus after MA administration, with peak values at 1 hr (Commins et al., 1985, submitted for publication), we have attempted to determine whether this endogenous 5,6DHT might mediate the hippocampal 5HT depletions seen after MA. This question was addressed in regard to the effects of AMT and PCPA on the long-term depletions. Rats were pretreated as indicated above with AMT or saline and then were given MA or saline in a latin square design. The animals were sacrificed 1 hr after MA administration. The amount of 5,6DHT detected in the hippocampi of the saline+MA group was 0.014 ± .006 ng/mg tissue (mean ± S.E.M., n=10). No 5,6DHT was detectable for the AMT+MA group, nor for either control group. Parallel studies are being carried out using PCPA.

Endogenous 5,6DHT is produced in rat hippocampus in the presence of MA. The results of this experiment raise the question as to whether this formation of 5,6DHT is an expression of an alternative metabolic pathway in the normal animal which may be accelerated in diseases of the central 5HT system. This research is supported by Mental Health Training Grant MH-14274; PHS DA-00250; L. Seiden is the recipient of an RSA Award MH-10562.

350.3 DOPAMINE UPTAKE INHIBITORS AND METHAMPHETAMINE NEUROTOXICITY. Gerard Marek* and Lewis S. Seiden. Dept. Pharmacol. & Physiol. Sci. University of Chicago, Chicago, IL 60637.

Previous studies have demonstrated that amfonelic acid, a dopamine (DA) blocker, was able to antagonize long-term depletion of striatal DA following a single amphetamine and iprindole injection (Fuller, R.W. and Hemrick-Luecke, Science 209:305(1980); Steranka, L., Brain Res. 234:123-136(1982) and multiple methamphetamine injections (Schmidt, C.J. and Gibb, J.W., Eur. J. Pharm. 109:73-80(1985). However, since amfonelic acid and other non-amphetamine stimulants seem to have effects on DA storage pools that might be independent of blocking the uptake of DA across the neuronal membrane (Shore, P.A., J. Pharm. Pharmac. 28:355(1976); Fuller, R.W., Perry, K.W., Bymaster, F.P. and Wong, D.T., J. Pharm. Pharmac. 30:197-198(1978), we are studying the effects of other DA uptake blockers such as benztropine, mazindol, and nomifensine as well as amfonelic acid on the neurotoxicity engendered by a large single injection (100 mg/kg s.c.) of methamphetamine (MA).

Amfonelic acid (10 mg/kg i.p.), Mazindol (20 mg/kg i.p.) or benztropine (25 mg/kg i.p.) were injected simultaneously with MA (100 mg/kg s.c.) or vehicle in male Sprague-Dawley rats of approximately 200-250 gms. Rats were sacrificed two weeks following these injections and the striata were subsequently assayed by reverse phase chromatography with electrochemical detection for DA, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), serotonin (5HT) and 5-hydroxyindole acetic acid (5HIAA). Amfonelic acid completely antagonized the MA-induced depletion of striatal DA, DOPAC and HVA. Mazindol partially protected against striatal DA depletions, but benztropine was ineffective. None of the DA uptake inhibitors antagonized the MA-induced neurotoxicity upon the serotonergic system.

Since it has been hypothesized that the MA-induced neurotoxicity is mediated via the formation of 6-hydroxydopamine (6OHDA) following massive release of DA into the synaptic cleft, (Seiden, L.S. and Vosmer, G., Pharmacol. Biochem. Behav. 21:29-31 (1984), it is of interest to compare the potency of DA uptake inhibitors in antagonizing intraventricular 6OHDA dopaminergic neurotoxicity vs. long-term MA-induced DA depletions. Preliminary evidence suggests dopamine uptake inhibitors exhibit a similar potency relationship for blocking ivt. 6OHDA neurotoxicity as they do for antagonizing MA-induced neurotoxicity. This research supported by PHS DA-00250; PHS 5T32 GM07281 MSTP; L. Seiden is the recipient of a RSA MH-10562.

350.4 **IN VITRO EFFECTS OF DOPAMINE ACTIVE COMPOUNDS ON MITOCHONDRIAL RESPIRATION AND OXIDATIVE PHOSPHORYLATION FROM RAT CORTEX, STRIATUM AND LIVER.**

J.H. Thakar,* M.N. Hassan* and J.D. Grimes.* (SPON:J. de la Torre) Parkinson Disorder Laboratory, Ottawa Civic Hospital, 737 Parkdale, Ottawa, Ontario Canada K1Y 1J8.

Neuroactive substances such as 6-hydroxydopamine (6OHDA), 1-methyl, 4-phenyl-1,2,3,6 tetrahydropyridine (MPTP) etc. have been used to evaluate the dopaminergic systems in animal models of movement disorders including parkinsonism. These are potent neurotoxic substances. For example, unilateral injection of 6OHDA in the substantia nigra of rodents produces parkinsonism. On the other hand MPTP is shown to produce parkinsonism in men, monkeys and mice. The exact mechanisms of action of these compounds at subcellular level are not yet well understood. We have investigated the effect of these compounds as well as dopamine (DA) and apomorphine (APO) on mitochondrial oxidative phosphorylation in vitro.

The mitochondrial preparations were isolated from cerebral cortex, neostriatum and liver. The homogenizing medium was made of 0.23M mannitol, 0.07M sucrose and 0.1mM EDTA at PH 7.4. The organelles were separated by differential centrifugation at 9,000g after removing the unbroken cells and cellular debris. The oxygen consumption and ATP synthesis were measured at 30°C using YSI oxygen monitoring system.

The preliminary observations in our study have shown that MPTP at concentrations of 0.1 to 0.4mM had little effect on respiration or on oxidative phosphorylation in organelles from striatum, cortex or liver. On the other hand, 6OHDA stimulated the oxygen consumption at concentrations above 10uM, without influencing the phosphorylation, i.e., ATP synthesis. Dopamine and APO did not have any significant effect on respiration and on oxidative phosphorylation at concentrations lower than 0.2mM. At higher concentrations, DA (0.7mM) and APO (0.45mM) produced some stimulation of oxygen consumption rates.

It is of interest to note that the stimulation of oxygen consumption produced by 6OHDA may be of significance in rendering its neurotoxicity. This particular property of 6OHDA has not been explored in any detail. Data on oxidative phosphorylation and respiration will be presented and discussed in detail.

(Supported by the Parkinson Disease Society of Canada).

350.5 **DOPAMINERGIC AGONISTS INDUCE IPSILATERAL ROTATION IN RATS AFTER INTRASTRIATAL INJECTIONS OF IMINODIPROPIONITRILE (IDPN).** J.L. Cadet* and W.J. Freed. Preclinical Neurosciences Section, Neuropsychiatry Branch, NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032.

**Introduction:** Chronic intraperitoneal injection of IDPN in rodents causes a persistent behavioral syndrome characterized by excitability, hyperactivity, and spasmodic dyskinetic movements which lasts throughout the lifespan of the animals. This syndrome is exacerbated by dopaminergic agonists and blocked by dopamine (DA) antagonists. Chronic treatment with IDPN leads to loss of both high and low affinity spiroperidol binding in the striatum (Diamond B, et al., Ann. Neurol. 12:97-98, 1982). In order to evaluate further the effects of IDPN on the DA system, we undertood this study on its influence on rotational behavior in rats.

**Materials and Methods:** Adult rats (175-200 g) were injected stereotaxically with saline or IDPN (250 $\mu$g in 10 $\mu$l, Eastman Kodak) into the right caudate-putamen using routine surgical techniques. Coordinates were 2 mm anterior to bregma, 2.5 mm lateral to midline, and 4.5 mm ventral to the surface of the skull. The animals were then challenged in random order with either apomorphine (1 mg/kg) or amphetamine (3 mg/kg). At least 2 days were allowed between testing. The results are expressed as means $\pm$ SEM.

**Results:** Both drugs induce significant ipsilateral turning behavior 5 to 25 days after IDPN treatment as compared to animals treated with saline: (APO: IDPN (49.1 $\pm$ 26.89, n=10); saline (4.01 $\pm$ .88, n=7, p .0005, t-test); (AMPH: IDPN (122.2 $\pm$ 27.05, n=10), saline (5.29 $\pm$ 1.48, p .0005, t-test). After 4-5 weeks, there was a reversal of the rotational behavior back to control levels.

**Discussion:** These results indicate that in the IDPN model both indirect (amphetamine) and direct (apomorphine) agonists influence rotation in a similar way, in contrast to rats with unilateral lesions of the substantia nigra. This suggests that IDPN interferes with dopamine-receptive neurons or DA receptors themselves, and causes an impairment of striatal function. Thus agonist agents stimulate the contralateral striatum and lead to rotation towards the side of IDPN infusions. This interpretation is consistent with other findings that intraperitoneal injection of IDPN causes a marked increase in HVA levels in the striatum (to be submitted), similar to the increase in HVA levels induced by neuroleptics.

350.6 STUDIES ON THE CAUSES FOR THE DOPAMINERGIC SELECTIVITY OF MPTP TOXICITY IN MICE. E.Melamed,J.Rosenthal*, O. Cohen*, M.Globus*, and A. Uzzan*. Dept. of Neurology, Hadassah University Hospital, Jerusalem, Israel.

The causes for the preferential toxic effect of MPTP on DA neurons are undetermined.Conversion of MPTP to MPP+ by MAO-B,is an important but probably not the only factor.MPTP DA toxicity may be dependent on additional biochemical processes unique for DA neurons.We therefore examined in mice the effects of various pharmacological manipulations of central DA metabolism including acceleration and suppression of DA turnover,DA depletion from nerve-terminals,and inhibition of DA uptake on the long-term decreases in striatal DA induced by MPTP.In C57 black mice,MPTP(30mg/kg,once daily x2,s.c.) produced marked falls in striatal DA levels which persisted up to 30 days after treatment.Mice were then given MPTP alone(30mg/kg x2, s.c.)or in combination with one of the following drugs(i.p.):haloperidol(2mg/kg),chlorpromazine(10mg/kg),apomorphine(5mg/kg),bromocriptine(2.5mg/kg),reserpine(0.1mg/kg),nomifensine,desipramine,clomipramine or fluoxetine(25mg/kg of each),amphetamine(5mg/kg)or L-dopa (50mg/kg with carbidopa 10mg/kg).All drugs were given for two days before,for two days in conjunction,and for two days after MPTP except for reserpine which was given for 4 days.Animals were decapitated 30 days after last injection.Enhancement(by haloperidol and chlorpromazine)or attenuation(by apomorphine and bromocriptine)of impulse flow in,and DA turnover by DA neurons did not affect decreases in striatal DA induced by MPTP.Likewise,depletion of vesicular DA from DA terminals by reserpine did not prevent toxic effects of MPTP.Nomifensine,a specific inhibitor of DA reuptake,completely abolished the MPTP-induced striatal DA depletions.By contrast,uptake inhibitors of NE(desipramine)or of 5-HT(clomipramine and fluoxetine)were ineffective.These findings suggest that MPTP(or a byproduct of its metabolism)is a substrate for the DA reuptake system and that its specific transport into nigrostriatal DA terminals may be a mandatory determinant for its selective DA toxicity.Cotreatment with amphetamine completely prevented MPTP toxicity. The protectivity of amphetamine is probably not linked to its effects on DA release(since reserpine was ineffective)but may be due to inhibition of DA reuptake and/or of MAO activity.Once inside the DA terminal,MPTP may cause neuronal damage via formation of free radicals such as superoxides.However,combined administration of MPTP with the antioxidants ascorbic acid and alphatocopherol and with dimethylsufoxide,a scavenger of hydroxyl radicals,did not affect MPTP toxicity.Autooxidation of L-dopa generates free radicals and it might augment toxic effects of MPTP.However,cotreatment with L-dopa partially suppressed MPTP-induced striatal DA reductions.Protective effect of exogenous L-dopa may be linked to competition of the formed and released DA molecules with MPTP on the DA reuptake system and/or on MAO-B.

350.7 NEUROTOXICITY OF SYSTEMIC MONOSODIUM GLUTAMATE IN THE ADULT RAT: ANALYSIS WITH SILVER STAIN DEGENERATION TECHNIQUES. S. Meharg,* T.T. Dinh* and S. Ritter. College of Veterinary Medicine. Washington State University, Pullman, WA 99164

Glutamate is an excitotoxic substance capable of destroying neuronal cell bodies when administered in high doses. Olney and his colleagues have established that systemic administration of monosodium glutamate (MSG) damages circumventricular organs (CVOs). Since glutamate does not readily enter the brain, it has been generally accepted that the toxicity induced by systemic administration of MSG is limited to circumventricular structures. However, the pattern of neuronal degeneration in the brain after systemic administration of MSG has not been fully analyzed. Since silver impregnation methods selectively label degenerating neurons, we felt that this technique might provide useful information regarding the neurotoxicity of systemic MSG for neural structures both within and outside the blood-brain barrier. Therefore, we injected adult female Sprague-Dawley rats with MSG (2 or 6 g/kg, s.c.) or equiosmotic concentrations of NaCl, sacrificed them 0, 6, 20, 36 and 120 hrs later, and prepared the brain tissue for microscopic analysis using a modification of the Carlsen-de Olmos cupric silver technique.

Neuronal degeneration was observed in MSG-treated rats, but not in NaCl-treated rats. Both doses of MSG produced marked degeneration with a similar anatomical distribution. As expected from previous work, MSG caused extensive degeneration within the CVOs. In the CVOs, the degeneration was most evident between 6 and 20 hrs after injection but was extensively present in these structures as soon as 20 min after MSG. In addition, axon and terminal staining, especially at the later time points, occurred in several structures known to receive afferent input from the subfornical organ. These sites include the bed nucleus of the stria terminalis, the nucleus medianus, the OVLT, and the paraventricular nucleus of the hypothalamus. Furthermore, discretely localized degeneration was present in a rather large number of sites throughout the brain, and the affected sites were highly reproducible across subjects. These sites include the dorsomedial aspect of the inferior colliculus, nuclei gracilis and cuneatus, the intercalated nucleus of the amygdala, endopyriform nucleus, and the accumbens.

Our silver stain degeneration studies confirm the results of other techniques regarding the neurotoxicity of MSG for circumventricular organs. Furthermore, they provide additional new data regarding the distribution of MSG effects throughout the brain.

350.8 GLIOTOXIN, α-AMINOADIPIC ACID, PROTECTS AGAINST KAINIC ACID NEUROTOXICITY IN THE STRIATUM. T. Hattori and M. Takada*. Dept. of Anatomy, Univ. of Toroto, Toronto, Ontario, Canada M5S 1A8.

Treatment with L-α-aminoadipic acid (L-α-AA), a six-carbon chemical analogue of glutamate, has been shown to cause degeneration of astrocytes in cell culture of dissociated mouse cerebellum. The specific toxic effect on astrocytic population was confirmed by indirect immunofluorescence labeling with anti-human glial fibrillary acidic protein antiserum (Huck et al., Neurosci., 12: 783, 1984).

At 1-3 day survival period following in vivo stereotaxic injections of 50-500 nmol L-α-AA in 1μl of 0.05M phosphate buffer (pH 7.4) into the rat striatum, clear and intense astrocytic degeneration was observed at the fine structural level. This phenomenon was quite specific in the sense that only astrocytes were destroyed. There was a considerable amount of cytoplasmic vacuolization in astrocytes, without any noticeable effect on neighboring neurons, oligodendroglia, microglia or endothelial cells.

Three days after coinjections of 2-5 nmol kainic acid (KA) and 50-125 nmol L-α-AA in 1μl of 0.05M phophate buffer (pH 7.6-8.0) into the striatum, the degree of KA destruction was significantly reduced as compared with the contralateral striatum, which received 2-5 nmol KA alone. D-isomer of α-AA neither caused astrocytic degeneration nor protected against KA neurotoxicity.

The present results indicate that astrocytic function plays a critical role in the early stages of KA induced degeneration in the rat striatum.

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350.9 PRENATAL EXPOSURE TO AMPHETAMINE OR RESERPINE ALTERS CONSUMMATORY BEHAVIOR IN ADULT RATS. R. Holson (SPON: J. Buelke-Sam), National Center for Toxicological Research, Division of Reproductive and Developmental Toxicology, Jefferson, AR 72079.

There is considerable evidence for central and peripheral monoaminergic modulation of food intake and body weight. Reserpine (R) and amphetamine (Amph) acutely affect monoaminergic neurotransmission and food intake in adults. If prenatal exposure to these drugs alters monoaminergic system development, then such alterations may be expressed in consummatory behavior. Moreover, since R and Amph have diametrically opposite effects upon central monoamines, any changes in consummatory behavior should be in opposite directions. To test this hypothesis, pregnant CD rats were given sc injections of Amph (3 mg/kg), R (0.375 mg/kg), or vehicle on gestational days 12 through 15. Two male and two female offspring from each litter were isolation-caged at postnatal day (PND) 70, and subsequently tested for: (1) baseline food and water intake (various ages); (2) intake of sweetened solutions (PND 80-90 and again after PND 210); (3) weight gain on a "junk food" diet (PND 100-120); (4) weight loss from quinine adulteration of water or restricted temporal food access (PND 127-133 and 145-155, respectively); and (5) acute anorexia caused by ip Amph or fenfluramine. Litter means were used as the statistical unit in all data analysis. It was found that prenatal Amph or R did not alter baseline body weight or food and water intake. Further, neither treatment altered weight loss under any of the above challenges. Prenatal R and Amph exposure did increase intake of sweetened solutions by 40%, (0.25% saccharin plus 3% glucose in particular) and this effect could still be seen on PND 210. R but not Amph exposure also elicited a 20% greater weight gain on the "junk food" diet. It is concluded that prenatal exposure to drugs which alter monoaminergic neurotransmission can elicit enduring changes in some measures of consummatory behavior.

350.10 STIMULATION OF CENTRAL ADRENERGIC RECEPTORS REDUCES CHOLINESTERASE INHIBITOR TOXICITY IN MICE. J.J. Buccafusco and R.S. Aronstam. Dept. Pharmacology and Toxicology, Medical College of Georgia and V.A. Medical Center, Augusta, GA 30912.

Recent studies in this laboratory have demonstrated that clonidine, a centrally-active $\alpha_2$-adrenergic agonist, affords protection against the toxic manifestations of physostigmine (J. Pharmac. Exp. Ther. 222, 595, 1982). This protection is mediated through stimulation of central $\alpha_2$-adrenergic receptors which decreases acetylcholine synthesis. This study was designed to determine whether stimulation of central adrenergic receptors affords protection against the irreversible inhibitor, soman (GD). ICR mice (25-34g) were injected s.c. with drugs administered in a volume of 5ul/g body weight. Lethal dose-response curves were constructed using 3-5 doses of GD. $LD_{50}$ values were obtained by linear regression analysis of log dose vs probit of % lethality plots ($r > 0.9$ in all cases). The LD curve for GD occurred over a very narrow dose range (0.1-0.2mg/kg) with an $LD_{50}$ of 0.156. Pretreatment with clonidine (CL) 5 min prior to GD afforded protection against the lethal effects of the cholinesterase inhibitor. Protection was maximal at 1mg/kg of CL. This dose shifted the LD curve for GD 1.25 fold to the right. Pretreatment with atropine (25mg/kg) also afforded protection against GD lethality, shifting the LD curve for GD 1.22 fold. When CL (1mg/kg) and atropine (25mg/kg) were combined in a pretreatment regimen, the LD curve for GD was shifted 1.8 fold. This shift for combined treatment is greater than that expected from the sum of the individual protective actions. Guanfacine (GF), a clonidine-like drug also inhibited the lethal effects of GD. The most effective dose of GF was 5mg/kg. Pretreatment with GF shifted the LD curve for GD 1.78 fold. In combination with atropine, GF shifted the LD curve for GD 2.33 fold. Methyldopa (MD) is a centrally-acting adrenergic agonist which is structurally dissimilar from CL. MD also is transformed within central neurons to its active metabolite, methylnorepinephrine. MD exhibited maximal protection against GD-induced lethality at 500mg/kg. Pretreatment with MD shifted the LD curve for GD 1.93 fold to the right. In combination with atropine, MD shifted the LD curve for GD 2.79 fold. For the 3 $\alpha_2$-adrenergic agonists tested, the order of effectiveness, alone, or in combination with atropine, was: MD > GF > CL. The order of potency was the reverse Combined treatment with atropine potentiated the protection afforded by the adrenergic agonists. These results support the validity of using combinations of pre-and post-synaptic inhibitors of cholinergic transmission to maximize protection from irreversible, organophosphate cholinesterase inhibitors. Supported by DAMD17-84-C-4117 and the Veterans Administration Medical Center.

350.11 GUANETHIDINE IS TOXIC TO NORADRENERGIC AND DOPAMINERGIC NEURONS IN CULTURES FROM RAT LOCUS CERULEUS AND SUBSTANTIA NIGRA. L. Friedman* and C. Mytilineou (SPON: M.D. Yahr), Department of Neurology, Mount Sinai Sch. of Med., New York, NY 10029.

Guanethidine is a ganglionic blocking agent which destroys the peripehral adrenergic neurons after in vivo administration to rats. Degeneration of sympathetic neurons occurs also in tissue culture, after treatment with 100 uM guanethidine, but only if the incubation medium is alkaline (pH 8.0) during the first 48 hrs of exposure to the drug (Wakshull et al., J. Cell Biol., 79:121, 1978). Intracranial injections of guanethidine have failed to induce toxicity to the neurons of the locus ceruleus.

We now report that guanethidine can be toxic to central norepinephrine (NE) and dopamine (DA) neurons in culture, at concentrations from 10 to 100 uM and physiological pH (7.2). Dissociated cultures were established from the dorsal pontine area (locus ceruleus) and ventral midbrain (substantia nigra) from fetal rat brains during the 14th day of gestation. From days 7 to 20 in vitro, cultures were treated with guanethidine at concentrations of 10 to 100 uM. Treated cultures were indistinguishable from controls by phase contrast microscopy during the entire period of treatment, indicating no generalized cytotoxic effect. On day 21 both control and guanethidine treated cultures were examined by CA histofluorescence after incubation with 10 uM alpha-methyl-NE for 30 min, to assure visualization of all CA neurons present. Exposure to 10 uM guanethidine resulted in reduced number of fluorescing neurons, while at 100 uM the cultures contained either none or very few faintly fluorescing neurons. The efficacy of guanethidine to use the CA uptake pump was tested by measuring the inhibition of 3H-NE and 3H-DA uptake in cortical and striatal synaptosomal preparations from adult rats. A potent inhibition was observed in the cortex (50% inhibition at 0.5 uM), while in the striatum inhibition occured only at guanethidine concentrations of 10 uM or higher. However, the cytotoxic effect of guanethidine in culture was similar to both NE and DA neurons.

It has been suggested (Manning et al., J. Neurosci., 3:714, 1983) that the sympathectomy produced by in vivo administration of guanethidine to rats results from an immune reaction of this animal species to the drug accumulated within the sympathetic neurons. Our results indicate that guanethidine can induce toxicity to central CA neurons in culture, via a mechanism that does not involve participation of the immune system. (Supported by NIH grant NS 18979).

350.12 A BEHAVIORAL TERATOGENIC INVESTIGATION OF THE ADRENERGIC AGONISTS CLONIDINE AND LOFEXIDINE IN THE RAT. W. J. Pizzi, R. Holson*, M. Giacinto* and S. Tarchala*. Division of Teratogenesis Research, National Center for Toxicological Research, Jefferson, AR and Department of Psychology, Northeastern Illinois Univ., Chicago, IL 60625.

Clonidine is a widely used antihypertensive agent with a potential for expanded therapeutic application in combating drug withdrawal symptoms in opiate and alcohol addiction. Recently, clonidine (CLO) and an analogue lofexidine (LOF) have been shown to block the opiate withdrawal syndrome in a variety of species, including man. One new application of these agents may be as an alternative to methadone maintenance with pregnant opiate addicts. Any consideration along these lines will require screening for reproductive outcome and behavioral teratogenic effects.

Dame were given s.c. injections of CLO (0.16 or 0.64 mg/kg) or LOF (0.64 or 2.56 mg/kg) once daily from GD 8-20. The doses employed were sufficiently high to cause a dose-related decrease in maternal weight gain. Pups also showed a decrease in birth weights which was still evident at PND-15 in all drug conditions. No significant differences were obtained on negative geotaxis, an auditory startle test, or a series of swimming immobilization tests across the periadolescent period. A series of activity measures carried out on PND-12, 14, 16, and 18 showed minor differences, probably related to decreased body weights in the high dose LOF male animals, but not in any of the other drug-treated groups.

A second set of experiments utilized clonidine and the ALZET osmotic minipump. On GD-9, three groups of animals were implanted s.c. with 14-day pumps, and CLO was infused at an initial dose of either 30, 60, or 120 mcg/kg/day. Three additional groups received the same doses via s.c. injections. Assessment of reproductive outcome and developmental landmarks was carried out, as well as administration of a series of developmental screening tasks for behavioral teratology (similar to those described above). No remarkable differences were seen on any of these measures. The offspring of an F-1 mating of these groups also failed to show reproductive or behavioral teratology.

Based on these developmental and behavioral data, along with normal litter sizes and survival rates, it is tentatively concluded that clonidine and lofexidine are not potent behavioral teratogens.

350.13 ORAL POLYCHLORINATED BIPHENYLS ELEVATE URINARY HOMOVANILLIC ACID: DETERMINATION OF CENTRAL CONTRIBUTIONS. K. O. Brosch* and R. F. Seegal. (Spon: J. Peck). Wadsworth Center for Labs and Research, N.Y. State Department of Health, Albany, NY 12201.

Occupational exposure to polychlorinated biphenyls (PCBs) induces neurological dysfunctions (Fischbein et al., Ann. NY Acad. Sci., 320:703,1979; Chia and Chu, Prog. Clin. Biol. Res., 137:117,1984) and experimental exposure induces neurochemical changes in laboratory animals (Agrawal et al., Toxicol. Lett. 7:417,1981; Seegal et al., Neurotoxicol., 6 :13,1985). We have reported (Seegal et al.,NSA, 8:83,1982; J. Tox. Envir. Hlth.,in press) that exposure to PCBs results in elevation of urinary homovanillic acid (HVA), a major metabolite of dopamine. We now report that the PCB-induced elevation in urinary HVA is due to increased central production of HVA.

Adult male rats were gavaged with either corn oil (controls) or corn oil containing Aroclors 1254 and 1260 at a final dose of 1000 mg/kg (approximately 1/10th the oral $LD_{50}$). 24h urine collections were made on alternate post-gavage days. On days 10 and 11, PCB and control animals received a single IP injection of debrisoquin sulfate (20 mg/kg). Debrisoquin is a peripheral monoamine oxidase inhibitor that does not cross the blood-brain barrier (Kendler et al., Eur. J. Pharm., 71:321, 1981) and results in suppression of peripheral HVA production. Thus, after treatment with debrisoquin, any HVA measured in the periphery should be of central origin. Concentrations of HVA were determined by high-performance liquid chromatography with electro-chemical detection. Results are expressed as µg HVA/24h.

PCB exposure led to a decrease followed by a prolonged elevation in urinary HVA. Debrisoquin induced a 20 µg/24h decrease in urinary HVA production in both groups. This reduction is approximately equal to 30% of the total HVA measured in the periphery and is similar to the percentage of peripheral over total HVA measured in the periphery by Kendler et al., (1981). The PCB/debrisoquin-treated animals continued to demonstrate a significant elevation in urinary HVA compared to corn-oil/debrisoquin controls. 48h after the last injection, both groups returned to their pre-injection HVA levels.

Thus, the continued elevation of HVA in the PCB/debrisoquin-treated animals is presumably central in origin and suggests that the PCB-induced elevation in urinary HVA is due to altered central factors rather than to increased peripheral production of HVA. These results strengthen the case for the measurement of peripheral concentrations of monoamine metabolites as useful indicators of central function.

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350.14 SEXUAL DIFFERENTIATION OF NEURAL AND NONREPRODUCTIVE BEHAVIORAL FUNCTION: EFFECT(S) OF DEVELOPMENTAL CHLORDECONE EXPOSURE. C.F. Mactutus, Lab. Behav. Neurol. Toxicol., NIH-NIEHS, Research Triangle Park, NC 27709.

The neurotoxic profile produced by developmental exposure to chlordecone has been previously shown to vary as a function of a number of factors including gender of the animal and prior test experience. While the estrogenic-like activity of chlordecone remains the putative explanation for the occurrence of its sex-dependent sequelae, little effort has been made to dissociate any enduring effects of this compound from its long half-life. The purpose of the present study was to determine the influence of developmental exposure to chlordecone on sexual differentiation with respect to neural and nonreproductive behavioral function. If chlordecone has an organizational effect on neural tissue, a neonatal day 4, but not a day 11 exposure should produce sexually-dimorphic alterations. If the consequences of chlordecone exposure, however, are more likely attributable to its presence in neural tissue (an activational effect), the alterations following day 11 exposure should be greater than those after day 4 exposure.

Fischer-344 rat pups, bred in our laboratory, were administered a single s.c. injection (20 µl) of either corn oil or of 1 mg/10 g chlordecone dissolved in corn oil. The pups of eight litters were injected on postnatal day 4, while the offspring of eight other dams were injected on day 11. Because of the potential influence of early and/or prior test experience, only one half of the pups of each litter were tested as preweanlings; all animals were tested upon maturation to adulthood. Early body weights were significantly depressed by the chlordecone, and to a greater extent by the earlier exposure. Consistent with an organizational effect, chlordecone induced a sexually-dimorphic alteration of adult body weight following the day 4, but not day 11, injection. An enhancement of the auditory startle response (day 20) by chlordecone as well as a long-term alteration in low-frequency body movement (day 120) also displayed sex-dependent effects following the earlier, but not later, exposure. On the other hand, chlordecone-induced tremor was significantly greater in animals exposed on day 11 than on day 4 (days 14 and 20). Other evidence consistent with an activational effect was noted on early measures of activity in a novel environment (day 15) and a lack of suppression of activity during the presentation of a novel tone (day 19). In no instance were long-term alterations detectable which were readily attributable to an activational effect, i.e., a persistent effect after the day 11, but not day 4, injection. Pronounced effects of prior test experience, however, were observed and interacted with the early chlordecone exposure. These latter findings suggested the choice of experimental design is an important determinant of the expression of subtle long-term functional alterations.

350.15 PROCONVULSANT AND ANTICONVULSANT PROPERTIES OF ISOMERS OF HEXACHLOROCYCLOHEXANE IN AMYGDALOID-KINDLED RATS. L.G. Stark, T.E. Albertson and R.M. Joy. Health Sciences Neurotoxicology Unit, Schools of Medicine and Veterinary Medicine, University of California, Davis, Ca. 95616.

Our previous studies have shown that treatment of rats with gamma-hexachlorocyclohexane (lindane) produces a dose-dependent increase in brain excitability which facilitates the acquisition and retention of kindled amygdaloid seizures. There is evidence from Matsumura and Tanaka (Cellular and Molecular Neurotoxicology, Ed. by T. Narahashi, New York, Raven Press, 1984, pp.225) that there may be important differences in the activity of the isomers of hexachlorocyclohexane (HCH) in insect muscle and rat superior cervical ganglion. The purpose of the work reported here was to compare several of the isomers of HCH with respect to their influence on the acquisition and expression of kindled amygdaloid seizures. In the first study, rats in 4 treatment groups (corn oil vehicle, or 5 mg/kg daily exposures to gamma-, beta-,or alpha-HCH) were given once-daily amygdaloid stimulations for 15 days and measurements of afterdischarge duration and seizure severity were recorded. The results confirmed previous work in the case of gamma-HCH showing proconvulsant activity and shorter times for acquisition of the kindled response. Alpha- and beta-HCH treated rats did exhibit delayed rates of kindling acquisition with the most notable difference in the beta-HCH group. The second study expanded the inital observations by comparing corn oil treatment with 3 dose-levels of exposure to the beta isomer (5,10 and 20 mg/kg). All rats were pretreated for two days before amygdaloid stimulations were begun and the combined treatment/stimulation protocol was continued for 12 more days. There was a dose-dependent decrease in the rate of acquisition of the kindled response due to the treatment with beta-HCH and both the average seizure severity and afterdischarge duration were diminished in a dose-dependent manner. Once treatment with the isomer was stopped, rats in the 3 groups did kindle with additional stimulations. These findings extend to whole brain the observation that significant differences do exist among the isomers of HCH with respect to their proconvulsant and anticonvulsant effects in the mammalian central nervous system.

350.16 GLUCOSE UPTAKE IN THE DEVELOPING RAT BRAIN UPON PRE- AND POSTNATAL EXPOSURE TO TRICHLOROETHYLENE. E.A. Noland-Gerbec*, R.J. Pfohl, D.H. Taylor*, and R.J. Bull§. Dept. of Zoology, Miami University, Oxford, OH 45056 and §College of Pharmacy, Washington St. University, Pullman, WA 99164.

Trichloroethylene (TCE) is a widespread contaminant of drinking water sources. The effects of TCE on glucose uptake by brain tissue were examined in pups from rat dams exposed to TCE in their drinking water. The pups were exposed throughout gestation and lactation. Glucose uptake in the cerebellum, hippocampus, and whole brain of pups during the first 21 days of life was measured with the $^{3}$H-labeled 2-deoxy-D-glucose dissection/scintillation counting technique. We determined that 312 mg TCE/l significantly depressed glucose uptake in the whole brains and cerebella of 7- to 21-day old pups. Glucose uptake was also lower in the hippocampus of exposed pups at 7, 11, and 16 days, but recovered to control levels by 21 days. No overt toxicity, such as lower body or brain weight, was observed at this exposure level.

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## OPIOIDS: PHYSIOLOGICAL STUDIES IV

351.1 THE EFFECT OF MORPHINE ON CELL SURVIVAL AND ACETYLCHOLINE METABOLISM IN THE CULTURED AVIAN CILIARY GANGLION. D.B. Gray, Dept. of Physiology and Neurobiology, Univ. of CT, Storrs, CT 06268.

Morphine, as well as enkephalins, is known to have neuromodulatory activity including postsynaptic hyperpolarization and presynaptic inhibition of transmitter release, both in vivo and in cultured ciliary ganglion neurons.[1] Since cultured ciliary ganglion cells contain enkephalin-like immunoreactivity,[2] it is possible that apiates may modulate transmitter release or synthesis at neuromuscular synapses. In order to determine if morphine had any effect on neuromuscular transmission in vitro, E9 chick ciliary ganglion neurons were dissociated and plated with E11 striated muscle cells. These cultures have been previously used as a sensitive measure of presynaptic ACh release at neuromuscular synapses,[3] by measuring ACh synthesis after high K+ (56mm) preincubation. In control co-cultures, neuronal ACh synthesis increased by >60% immediately after high K+ stimulation in order to compensate for transmitter release (not seen in cultures of neurons alone). When 5 µm morphine was added to the high K+ preincubation, ACh synthesis rates did not increase but decreased by 17%. This effect appears to be mediated through a presynaptic opiate receptor since addition of both morphine and naloxone (at 5 µm) to the preincubation solution restores the compensatory increase in ACh accumulation. In support of this data, addition of 5 µm morphine to co-cultures significantly reduces the total recoverable ACh secreted into the incubation solution follwoing stimulation by high K+ Tyrodes.

Effects of chronic administration of morphine were also examined since it is known that daily injection of opiates can delay cell death in the developing chick ciliary ganglion (Meriney & Pilar, this volume). In cultures of E9 neurons alone, addition of chick embryo extract is necessary for survival past 2-3 days. In order to determine whether a classic opiate can replace chick embryo extract in vitro, growth media containing morphine sulphate (5 to 50 µm), but not chick embryo extract, was given to E9 chick embryo ciliary ganglion neurons plated upon dried collagen-coated microwells. More than 80% of neurons died within 72 hours after plating, and showed no significant difference from cultures without either extract or morphine. Thus it is clear that morphine cannot replace chick embryo extract in these cultures. These results appear not to be due to a toxic effect of morphine since cultures grown in media with both 10% embryo extract and 50 µm morphine are normal in appearance and number over all times examined. Supported by BSN 8410581.

[2] Crean, G., M. Ogawa, & G. Pilar; 1984 Soc. Neuro. Abs. 10:#127.15
[3] Gray, D.B & J.B. Tuttle; 1984 Soc. Neuro. Abs. 10:#307.10
[1] Margiotta, J.F. & D.K. Berg; 1984 Soc. Neuro. Abs. 10:#239.5

351.2 INCREASES IN MEDIAN EMINENCE GLUCOSE UTILIZATION AND SUPPRESSION OF PLASMA PROLACTIN LEVELS BY NALOXONE IN RATS. R.C. Walovitch, M.K. Selmanoff, F. Snyder* and E.D. London. Neuropharmacology Lab., NIDA Addiction Research Ctr., Baltimore, MD 21224 and Dept. of Physiology, Univ. of Maryland, Sch. of Medicine, Baltimore, MD 21201.

Opioids stimulate prolactin secretion in mammals (Shaar, C.J. and Clemens, J.A., Fed. Proc., 39:2539, 1980). This effect is suppressed by opioid antagonists, which alone can decrease basal levels and stress-induced elevations of prolactin (Blank, M.S. et al., J. Endocrinol., 85:307, 1980; Ragavan, V.V. and Frantz, A.G., Life Sci., 28:921, 1981). The opioid-induced increase in prolactin secretion may be mediated by inhibition of dopamine release from the tuberoinfundibular dopaminergic (TIDA) neurons (Gudelsky, G.A. and Porter, J.C., Life Sci., 25:1697, 1979).

In order to study further the relations between endogenous opioid systems, the activity of TIDA neurons and prolactin secretion, we administered naloxone (1, 10, or 20 mg/kg, iv) to 4-6 months old male Fischer-344 rats, and measured plasma prolactin levels and glucose utilization in the arcuate nucleus and median eminence, brain regions which contain the TIDA neuronal cell bodies and terminals, respectively. Plasma prolactin concentrations were measured using a double antibody radioimmunoassay in samples taken prior to, and at 5, 10, 15, and 30 min after naloxone administration. Glucose utilization was measured using the 2-deoxy-D-[1-$^{14}$C]glucose ([$^{14}$C]DG) method (Sokoloff, L. et al., J. Neurochem., 28:897, 1977). All animals used in the study were partially immobilized for 3-4 hr and received [$^{14}$C]DG (125 µ Ci/kg, iv) 5 min after placebo or naloxone.

Mean plasma prolactin concentrations doubled during the first 15 min following the placebo injection. This increase was attributed to stress associated with handling of the rats and blood sampling before and during the [$^{14}$C]DG procedure. At doses of 1 mg/kg or 10 mg/kg, naloxone reduced the stress effect on plasma prolactin; whereas, 20 mg/kg naloxone reduced prolactin levels by 40% below the baseline at all times sampled. Glucose utilization in the arcuate nucleus was unaffected by naloxone treatment. In contrast, all doses of naloxone increased glucose utilization in the median eminence by approximately 30%. The increase in glucose utilization of the median eminence is consistent with a blockade by naloxone of the tonic inhibition of TIDA neurons by central opioid systems. The metabolic activation of the median eminence also may reflect an opioid modulation of other neurotransmitter systems which project to the median eminence. (Supported in part by a fellowship from E.I. DuPont De Nemours and Co. to RCW)

351.3 THE HOT PLATE, TAIL-FLICK AND ABDOMINAL CONSTRICTION (WRITHING) TESTS OF ANALGESIA ARE NOT MEASURING THE SAME PHENOMENA. J.K. Belknap, P.W. Danielson* and J. Buegel.* Dept. of Pharmacology, Univ. of North Dakota, School of Medicine, Grand Forks, ND 58202.

Mice were selectively bred to be either highly sensitive (high response line) or highly resistant (low response line) to the antinociceptive effects of i.p. levorphanol (a morphine-like analgesic) on the hot plate assay (52.5°C). Over each of seven generations, all animals were hot plate-tested (N=200-260) and the highest scoring quartile of the high response line and the lowest scoring quartile of the low response line were bred to produce each succeeding generation in the high and low lines (lineages), respectively. This resulted in about a 3-fold difference between the lines in the i.p. or i.c.v. dose of levorphanol (and also morphine) required to produce an equivalent degree of analgesia in Generation 7. Since all of these animals were raised and tested under equivalent conditions, these differences between the high and low response lines are genetic in origin. These selection lines differ only slightly in hot plate latencies following saline injections, and they do not differ at all in brain levorphanol or morphine concentrations following equivalent i.p. doses of either narcotic analgesic.

We sought to determine if these large differences (∿300%) in analgesia bred into the high and low response lines with respect to the hot plate assay would generalize to two other commonly used tests of analgesia, namely the tail-flick and abdominal constriction tests. The tail-flick test was administered 20 min following a single 0 - 2.0 mg/kg i.p. dose of levorphanol tartrate. Only a 10-25% difference between the high and low lines was seen in either latency to respond or in the temperature at which a tail-flick occurred. The abdominal constriction test was administered in separate groups of mice by counting the number of abdominal constrictions following 0.6% acetic acid i.p. and 0-.8 mg/kg levorphanol tartrate s.c. Only small differences (20-40%) between the high and low lines were seen.

In sum, animals selectively bred to show either very high and very low analgesic responses on the hot plate assay showed rather small differences (although in the expected direction) when tested on two other tests of analgesia. These data suggest that the mechanisms causing the hot plate assay differences between highs and lows are largely different from those operating with the other two tests of analgesia. Thus, there appears to be little commonality in the mechanisms underlying hot plate-assessed analgesia vis a vis the tail-flick and abdominal constriction assays. (Supported by PHS Grant DA 02723)

351.4 INCREASED ADENYLATE CYCLASE ACTIVITY IN MOUSE SPINAL CORD-DORSAL ROOT GANGLION(DRG) EXPLANTS RENDERED TOLERANT BY CHRONIC EXPOSURE TO MORPHINE. B.Dvorkin*, S.M.Crain#, and M.H.Makman (SPON:E.J.Simon). Depts. of Biochemistry, Neuroscience# and Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

Acute exposure of fetal mouse cord-DRG explants to morphine and other opioids results in naloxone-reversible depression of DRG-evoked dorsal-horn synaptic-network responses. After chronic opiate exposure (1uM) for 2-3 days, these dorsal cord networks become tolerant even to much higher opioid concentrations (Crain etal ´79). A similar "acute tolerance" to opioids is elicited by brief exposure of explants to forskolin or high levels of cyclic AMP (Crain etal ´84), suggesting that tolerance following chronic exposure to opioids is mediated by enhancement of the adenylate cyclase/cyclic AMP (AC/cAMP) system (e.g. Sharma etal´75).

For measurements of AC, 6-8 fetal mouse cord-DRG cultures (ca. 3 wks in vitro) were pooled and homogenized for assay of the particulate(membrane) fraction. AC activity of control homogenates was stimulated by addition of forskolin (+204%) and naloxone (+68%); forskolin-stimulated AC was inhibited by levorphanol but not by dextrorphan. Chronic exposure of cord-DRG cultures to 1uM morphine for 4-7 days, starting at 2 wks in vitro, resulted in enhanced basal (+46%), as well as forskolin-stimulated (+70%), and naloxone-stimulated (+68%) AC activity; also, levorphanol no longer inhibited forskolin-stimulated AC.

There is increasing evidence that opioid effects on AC are mediated by a GTP-regulatory protein, $G_i$, and that these effects are blocked by pertussis toxin(PT)-induced ADP-ribosylation of $G_i$. In preliminary experiments, treatment of cord-DRG cultures for 5 days with PT (1ug/ml) led to enhancement of basal- and forskolin-stimulated AC, comparable in magnitude to that produced by chronic morphine; also, opioid inhibition of forskolin-stimulated AC was greatly diminished. It is postulated that $G_i$ normally mediates tonic inhibition of AC by endogenous opioids and/or other modulators in cord-DRG cultures. This interpretation is supported by the ability of naloxone to stimulate AC (see above) and to enhance dorsal-horn responses (Crain etal ´77) in control cultures. Chronic opioid exposure may then increase tonic inhibition of AC by endogenous opioids, which in turn results in compensatory enhancement of the AC/cAMP system.

Since opioid-sensitive AC and opioid receptors are widely distributed in cord-DRG cultures, further studies are required to determine if chronic opioid treatment induces specific alterations in AC activity and cAMP levels of the dorsal-horn regions which result in the physiologic expression of tolerance. (Supported by research grants NS-09649 & AG-00374 to M.H.M., & DA-02031 to S.M.C.)

351.5 Ovarian Steroids Decrease the Hypothermic and LH Responses to Morphine L.A. Berglund*, W.R. Anderson, M.J. Katovich and James W. Simpkins, Department of Pharmacodynamics, College of Pharmacy, University of Florida, Gainesville, FL 32610.

Studies were undertaken to determine the effects of an ovarian steroids regimen, which induced a surge in LH secretion, on the ability of morphine to suppress LH release and to induce hypothermia in the female rat. Two weeks after ovariectomy, rats received no steroids (OVX) or a steroid regimen consisting of 7.5 ug estradiol benzoate s.c. in oil at 1000 h and 5 mg progesterone, s.c. in oil 48 hours later. In the first experiment, serum LH concentrations were determined at 1600 h, 30 min after the administration of saline or 7.5 to 20 mg morphine sulfate/Kg BW. In OVX rats, morphine caused a dose-dependent decrease in serum LH concentrations. (Serum LH = 5.8± 0.8 ng/ml for saline; 2.8±0.4 ng/ml for 7.5 mg morphine/Kg BW, and 1.4±0.3 ng/ml for 10.0 mg morphine/Kg BW). In contrast, morphine, at doses as high as 20 mg/Kg BW, did not significantly alter serum LH levels during the steroid-induced LH surge at 1600 h. (Serum LH = 51.4±8.7 ng/ml for saline; 53.1±9.7 ng/ml for 10 mg morphine/Kg BW: and 49.8±10.8 ng/ml for 20 mg morphine/Kg BW). In a second experiment we determined if also the hypothermic effects of morphine were altered by steroid treatment. OVX rats were treated with gonadal steroids as indicated above and were compared to OVX rats which received no steroid treatment. At 1630 h, animals were lightly restrained, fitted with rectal thermistor probes and allowed 0.5h to acclimate. Then rats were administered morphine sulfate (20 mg/Kg BW, sc) and rectal temperatures were monitored at 2-5 min intervals for an additional 3 h. In OVX rats, morphine caused a 1.85±0.35° C decrease in rectal temperature. In contrast, in rats treated with steroids, the morphine-induced decline in rectal temperature was 0.64±0.2°C. Collectively, these data indicate that a gonadal steroid regimen, which is stimulatory to LH secretion, abolishes the capacity of morphine to suppress LH release and to lower core body temperature. It would appear that the brain opiate receptors which mediate the effects of morphine on LH secretion and body temperature are rendered insensitive during the steroid-induced LH surge. This may contribute to the series of neuronal events which mediate steroid-induced LH hypersecretion and the change in core body temperature associated with ovulation. (Supported in part by NIH grants AGO2021 and HD 18133).

351.6 THE EFFECTS OF RESTRAINT AND NALTREXONE ON THE HEART-RATE RESPONSE TO MORPHINE. K.S. Schwarz*, J. Peris, * & C.L. Cunningham* (SPON. J. O'Brien). Dept. of Medical Psychology Oregon Health Sciences Univ., Portland, OR 97201

Previous reports about the heart-rate effects of morphine in rats suggest that only a decrease in heart rate (bradycardia) occurs following intravenous (i.v.) administration. However, our studies show that when a freely-moving rat is allowed time to habituate to the apparatus, morphine causes a transient bradycardia followed by a large increase in heart rate (tachycardia).

In each experiment, two cardiac electrodes and one jugular-vein cannula were surgically implanted into male albino rats a few days before the start of testing. During all test sessions, rats were put into chambers 1 hr before i.v. morphine infusion and remained there for 2 more hrs. Heart rate was recorded for each minute of each session.

Experiment 1 assessed the effects of five doses (0, 0.5, 2.0, 5.0 & 10.0 mg/kg) of morphine in restraint-stressed and freely-moving rats. The restraint condition elevated baseline heart rate (406 vs 353 bpm). Overall, morphine resulted in a biphasic response: bradycardia followed by tachycardia relative to pre-infusion baseline. Both the magnitude and duration of bradycardia were enhanced by restraint. In freely-moving rats, the initial bradycardia was quickly replaced by a longer-lasting, greater-magnitude tachycardia. These effects increased as dose increased. These results suggest that the level of stress may be an important factor in determining the predominant direction of the heart-rate response to morphine.

Experiments 2 & 3 attempted to determine if the bradycardia and tachycardia were direct morphine effects. Only freely-moving rats were used. In Experiment 2, saline or naltrexone (5 mg/kg) was injected subcutaneously before placement into the chamber. Naltrexone blocked both the bradycardic and tachycardic reactions of the 8 mg/kg dose of morphine. These results suggest that both the decrease and increase are direct morphine effects; however, we could not eliminate the possibility that the increase in heart rate was simply dependent on the occurrence of the initial bradycardia. We tested this in Experiment 3 by delaying the infusion of naltrexone until after morphine-induced bradycardia had occurred. Specifically, morphine (8 mg/kg) was infused i.v. and was followed 45 min later by an i.v. infusion of saline or naltrexone (5 mg/kg). We conclude that both the bradycardia and tachycardia are opiate-mediated and, furthermore, that the tachycardia is independent of the bradycardia.

351.7 INVOLVEMENT OF THE CENTRAL SEROTONERGIC SYSTEM IN MORPHINE-INDUCED INHIBITION OF TSH RELEASE IN THE RAT. W.J. Litto and J. Rabii. Dept. of Biological Sciences and Bureau of Biological Research, Rutgers Univ., Piscataway, NJ 08854

It has been shown that morphine (MS), acting at a hypothalamic site, inhibits TSH release in the rat. Although the exact mechanism by which MS inhibits TSH release has not been determined, the involvement of biogenic amines in this action has been postulated. In the present study we have looked at the effects of pharmacological and surgical manipulations of the central serotonergic system on the ability of MS to inhibit TSH release in adult male rats. Animals in these experiments were implanted with chronic right atrial cannulae, under light ether anesthesia, at least 48 hours prior to MS challenge (intravenous). In the first experiment, animals were treated with either p-chlorophenylalanine (PCPA; 250 mg/kg IP), or saline. Forty-eight hours later a blood sample was withdrawn followed by a MS injection (7 mg/kg). Subsequent blood samples were then withdrawn 30 and 60 min after MS injection. In a second experiment, groups of animals were subjected to either dorsal raphe nucleus (DR) lesions (electrolytic), or were sham-operated. One week following surgery, an initial blood sample was taken followed by 1 mg/kg MS. Subsequent blood samples were withdrawn 15, 30, and 60 min following MS injection. In a third experiment, following an initial blood sample, animals were treated with SQ-10631 (10 mg/kg) or vehicle. Sixty min following this treatment, a second blood sample was taken, followed by MS injection (1 mg/kg). Subsequent blood samples were then taken 30 and 60 min following MS treatment. In all experiments, blood samples were centrifuged and the plasma collected and stored frozen until assayed for TSH by RIA. In the first experiment, MS produced significant inhibition of TSH release in the vehicle-treated animals, but did not effect TSH release in PCPA-treated rats. In the second experiment, MS produced significant declines in TSH at all times tested in the sham-operated group, but had no effect on TSH in the DR-lesioned animals. In the third experiment, pretreatment with SQ-10631 eliminated the inhibition of TSH produced by MS that was observed in the vehicle pretreated group. In conclusion, the inhibition of TSH release produced by MS may involve the central serotonergic system. (Supported by the Busch Memorial Fund.)

351.8 FURTHER CHARACTERIZATION OF THE TEMPORAL EFFECTS OF MORPHINE ON PROLACTIN RELEASE IN MALE RATS. J. Janik*, P. Callahan*, L. Grandison and J. Rabii. (SPON: M.T. Spoerlein). Dept. Bio. Sci., Rutgers Univ. and Dept. Physiol., UMDNJ, Piscataway, N.J. 08854.

We have previously demonstrated that a single injection of morphine has temporal effects on the tuberoinfundibular dopaminergic neurons and that these effects are reflected in the prolactin secretory response. Treatment with morphine sulfate (MS) initially stimulates prolactin release but by 4 hours it attenuates the response to physiological and neuropharmacological stimuli of this hormone. This study was designed to further characterize the delayed, inhibitory effects of MS on prolactin release. First we examined the dose of MS which would attenuate a subsequent MS induced prolactin secretory response 4 hours later. Animals received 2.5, 5, 10, 15 or 20 mg/kg,sc MS and the prolactin secretory responses to the initial and second (challenge) injection were determined. Blood samples were obtained immediately before and 30, 45 and 60 minutes after each injection. At all doses of MS, the prolactin secretory response to the challenge injection was attenuated. MS at 2.5 and 5 mg/kg produced partial attenuation and doses of 10 and above produced complete inhibition of the prolactin response. However, the GH response to the challenge injection was not attenuated in these rats. Next, we examined whether or not the delayed inhibitory effect of morphine could be blocked by the opiate receptor antagonist naloxone. When naloxone (2 mg/kg,ip) was administered before the initial MS injection (15 mg/kg,sc), the prolactin secretory response to the challenge was no different from controls. In conclusion, MS produces a biphasic effect on prolactin secretion. There is an initial stimulation of release but by 4 hours a subsequent stimulus induced prolactin increase is blunted. These observations indicate that the delayed (4 hour) response is an opiate receptor mediated process (i.e. naloxone reversible). Furthermore, this attenuated response to the challenge is produced by morphine in a dose dependent manner. (Supported by the Busch Memorial Fund.)

351.9 ALTERATIONS IN THE SENSITIVITY OF THE PROLACTIN REGULATORY MECHANISM TO OPIATES DURING LACTATION. P. Callahan*, J. Janik*, L. Grandison and J. Rabii. Dept. Bio. Sci., Rutgers Univ., and Dept. Physiol., UMDNJ, Piscataway, N.J. 08854.

It is well established that opiate administration produces a significant increase in circulating prolactin and GH levels in male and non-lactating female rats. One of the mechanisms involved in opiate induced prolactin release is inhibition of the tuberoinfundibular dopaminergic (TIDA) neurons. We have previously reported that the morphine induced prolactin increase is not observed in lactating female rats after 2 hours of pup separation when circulating prolactin is maintained at low levels. We have attempted to further characterize the sensitivity of the lactating female model to opiate stimulation. Female rats, between 4 and 10 days postpartum, received a single injection of saline or morphine sulfate (5 mg/kg,iv or 10 or 15 mg/kg,sc). Blood samples were withdrawn and assayed for GH. In contrast to the lack of a stimulatory influence of morphine on prolactin release, a single injection of morphine produced a significant increase in GH levels at all doses. It was of interest to determine whether or not the lack of a morphine induced prolactin increase was due to the physiological state of the animal, i.e. suckling. When animals received saline on day 6 postpartum and continued nursing their pups through day 10, there was no increase in circulating prolactin levels in response to a single injection of morphine (15 mg/kg,sc) on day 10. Similarly, if animals received MS on day 6 and continued nursing, a morphine induced prolactin increase was not observed on either day 6 or 10. Animals on day 6 postpartum did not show an MS induced prolactin response following 2 hours of pup separation. However, on day 10, after 4 days of pup separation, 50% of the animals in this group showed a morphine induced prolactin increase. This is the only group in which any increase in prolactin was observed. There may be a period of recovery which is necessary before a morphine induced prolactin increase can be observed. These results indicate that the lactating female model is insensitive to morphine stimulation of prolactin, but not GH release. They also suggest that this lack of sensitivity is due to the physiological state of the animal, i.e. suckling. (Supported by the Busch Memorial Fund).

351.10 DISTINCT REPONSES TO LEU-ENKEPHALIN AND FMRFAMIDE ON IDENTIFIED NEURONS OF THE APLYSIA CEREBRAL GANGLION. K. S.-Rozsa* and D. O. Carpenter. NY State Dept. of Health, Albany, NY 12201 and Biol. Res. Inst., Hungarian Acad. Sci., Tihany, Hungary.

Neurons of the B cluster of the cerebral ganglion of Aplysia were recorded with two electrodes under current or voltage clamp and responses examined to leu-enkephalin ($10^{-4}$ M to $10^{-3}$ M) and FMRFamide ($10^{-5}$ to $10^{-4}$ M), applied by brief pressure pulses. Both substances induced a variety of responses on different neurons. By careful dissection and drawings of the ganglia it was possible to regularly identify 16 different neurons on each side within the cluster, and demonstrate a consistent response to each of the peptides on single identified cells. Of the 16 neurons in each cluster which could be identified more than half responsed to leu-enkephalin. Of the responsive neurons about half were depolarized, a quarter were hyperpolarized and the remainder showed biphasic or polyphasic responses. The depolarization was associated with an increase in membrane conductance and was reduced when $Na^+$ was replaced with choline or glucosamine. The hyperpolarizing response was slow (lasting usually 30-60 sec), was depressed by 5 mM 4-aminopyridine and sometimes could be reversed near to the $K^+$ equilibrium potential. The responses to leu-enkephalin often showed rapid and long-lasting receptor desensitization. FMRFamide elicited a depolarizing response on most of the B cells, but some neurons did not respond and some showed hyperpolarization or multiphasic responses. FMRFamide responses did not desensitize as readily as those to leu-enkephalin. While the responses to the two peptides had similar appearances, some neurons were depolarized by one and hyperpolarized by the other, indicating distinct receptors. The leu-enkephalin response was sensitive to naloxone, but only at relatively high concentrations ($5X10^{-3}$ M) which depressed the FMRFamide response to a lesser degree. These results support a possible transmitter function for both classes of peptides in Aplysia ganglia.

351.11 EFFECT OF MORPHINE ON ENKEPHALIN RELEASE. Y.Y.T. Su. Cullen Eye Institute, Baylor College of Medicine, Houston, Texas 77030.

We have recently shown that exogenously applied enkephalins inhibit $^3$H-GABA release from GABAergic amacrine cells of the chicken retina. Anatomical localization studies, using double staining techniques, further demonstrated that some GABAergic amacrine cell populations also contain enkephalin immunoreactivity (Watt et al., Nature, 311:761, 1984). These results suggested that the enkephalins may inhibit $^3$H-GABA release from those amacrine cells containing only GABA and those containing both GABA and enkephalins. In this report we show the effect of morphine on enkephalin release from the retina.

The newly synthesized enkephalins in chicken retinas were labeled with $^3$H-methionine by a pulse-labeled and chase technique described earlier (Su et al., J. Neurosci., 5:851, 1985). Briefly, the isolated retinas were incubated in oxygenated avian Ringer's solution (200 µl) containing 50µCi $^3$H-methionine (specific activity = 80 Ci/mmol) for 2 hrs. After 3 washings, the tissues were incubated in the oxygenated Ringer's solution containing methionine (0.1mg/ml) for another 2 hrs. The tissues were then utilized to study $^3$H-Met$^5$-enkephalin release.

The labeled tissues were incubated in high $K^+$ (30 mM) Ringer's solution with or without (as control) 1µM morphine, followed by several washings in normal Ringer's solution. After the release experiment the soluble radioactively-labeled compounds were extracted in 2 N acetic acid. The tissue extract was lyophilized and resuspended in PBS. Six hundred microliters of the eluent and the tissue extract were mixed with 100µl of a monoclonal anti-enkephalin IgG in ascites fluid (1:20 dilution in PBS) and incubated overnight at 4°C. The antigen-antibody complexes were precipitated with equal volume of saturated ammonium sulfate solution and resuspended in 200µl of water. The activity was measured in a scintillation counter. $^3$H-Met$^5$-enkephalin released was expressed as percentage of the total enkephalin-immunoreactivity present in the tissue.

In the presence of 1µM morphine, the high $K^+$-induced $^3$H-Met$^5$-enkephalin release was reduced to 62% of the control. These results suggest that some opiate receptors may be present on the enkephalinergic amacrine cell processes and that morphine may function as a presynaptic inhibitor as it binds to these opiate receptors. However, the inhibition induces from an indirect serial synaptic interactions can not be excluded. More studies are required to elucidate the mechanisms of the inhibition of enkephalin release.

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351.12 EFFECTS OF ENKEPHALINES ON DARK ADAPTATION IN ISOLATED RETINA OF CRAYFISH. J. Flores*, P. Freijo*, I. Marquez* and N. Blazquez* (SPON:J.Hernandez). Dept. of Neurosciences, ENEP Iztacala UNAM. Los Reyes Iztacala Ap. Post. 314-54030 Edo. de Mex., México.

The presence of leucine-enkephalin (leu-enk) has been demonstrated with immunocytochemical methods in the eyestalk of Palinurus interruptus and Procambarus clarkii. It is localized in retinular cells, lamina ganglionaris, medulla interna, third optic chiasma and medulla terminalis (Mancillas, J. P., Mc. Cinty, J. F., Selverston, A. I., Karten, H. and Bloom, P. E. Nature 293:576-578, 1981). The influence of enkephalines on visual function is not clear. The aim of this study is to evaluate the possible role of leu-enk and methionine-enkephalin (met-enk) on dark adaptation.

Experiments were conducted in adult crayfishes Procambarus clarkii of either sex in C or D stage of molting cycle. The control retinas were left on physiological saline and the experimental groups on leu-enk, met-enk and naloxone salines ($10^{-6}$M to $10^{-4}$M). Electroretinograms were recorded on constant dark, using suction electrodes. Light pulses were delivered from an incandescent source (aproximate intensity of 3.16 candle/ft$^2$ and duration of 100 msec) each 3 minutes.

Leu-enk and met-enk incubated retinas showed a significant increase in the rise time of the dark adaptation curve. The effect was blocked by naloxone ($10^{-5}$M). These results suggest a possible opioid effect on dark adaptation processes.

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351.13 STEREOSPECIFIC EFFICACY OF THE OPIATE ANTAGONIST WIN44,441-3 IN THE TREATMENT OF HEAD INJURY IN THE CAT. T.K.McIntosh, V.M.Agura* M.Hellgeth‡ H.Rittner‡ A.I.Faden and R.L.Hayes. Dept. of Neurology, Univ. California Med Cntr., San Francisco, CA 94143 and Division of Neurosurgery, Medical College of Virginia, Richmond, VA 23298.

Traumatic injury to the central nervous system (CNS) may cause functional deficits through the release of endogenous factors, including endogenous opioids. Antagonism of such factors may therefore serve to limit the extent of injury, thus improving outcome. We have provided evidence that the kappa receptor and the dynorphin opioid system may play a pathophysiological role in traumatic spinal injury. Moreover, the non-selective antagonist naloxone has been found to improve several physiological parameters after fluid percussion (FP) head injury in cats. The present study compared the effectiveness of the opiate antagonist WIN44,441-3(-) (which has enhanced activity at the kappa receptor) with its inactive stereoisomer WIN44,441-2(+) in FP head injury in cats. FP simulates a number of features of concussive brain injury in humans including systemic hypotension and alteration in cerebral blood flow (CBF). Cats (2.75-3.25kg) were anesthetized with brevital (20mg/kg), intubated, paralyzed with pancuronium (0.6mg/kg) and artificially ventilated with 70%$N_2$O/30%$O_2$. Mean arterial pressure (MAP) and intracranial pressure (ICP) were monitored via a femoral arterial catheter and epidural bolt, respectively. 15 minutes following FP (3.2-3.6atm), animals were randomly assigned to treatment with an IV bolus of either WIN(-) (0.2mg/kg,n=5) or its inactive stereoisomer WIN(+)(0.2mg/kg,n=4). Sequential measurement of CBF using radiolabeled microspheres was performed 10 min prior to injury, 15 min after injury (immediately prior to drug administration) and 30 min, 1h and 2h following drug treatment.

Following a brief hypertensive event (1-3min), FP induced a significant decrease in MAP (from 150±7 to 104±8mmHg, $p < 0.05$) and whole brain blood flow (from 52±12 to 40±6ml/100g/min, $p < 0.05$). No significant change was observed in ICP. Administration of WIN(-) caused a significant increase in MAP within 5 min (mean incr=55mmHg $p < 0.05$) that was maintained for up to two hours with a concomitant increase in whole brain blood flow ($p < 0.01$). Blood flow was significantly increased to the midbrain ($p < 0.05$), brainstem ($p < 0.01$) and cerebellar regions ($p < 0.05$) where injury, evidenced by histological changes, was most severe. WIN(-) also caused a small but nonsignificant increase in ICP. Administration of WIN(+) was without effect on MAP and CBF which continued to decline over the two hour period. These results suggest that endogenous opioids, through actions at specific opiate receptors, contribute to the pathophysiology of head injury and indicate that opiate antagonists with increased activity at kappa sites may be effective in the treatment of low flow states associated with acute head injury.

351.14 IRRADIATION MODIFIES THE ANALGESIC EFFECTS OF MORPHINE. J. Aronowski*, P.M. Dougherty*, T. Samorajski, N.R. Pellis and N. Dafny (SPON: P.T. Kelley). Department of Neurobiology and Anatomy and Department of Surgery and Organ Transplantation, University of Texas Medical School and Texas Research Institute of Mental Sciences, Houston, Texas 77030.

Previous studies using the immunomodulators, α-interferon, cyclosporine A and cyclophosphamide) prior to and after subcutaneous implantation of a 75 mg morphine pellet demonstrated that the immunomodulator agents altered the opiate addiction liability as assessed by naloxone induced abstinence in morphine addicted rats. Moreover, selective ablation of the immune system via whole body irradiation of 500 rads prior to and after chronic morphine treatment had the same effect as α-interferon, cyclosporine A and cyclosphosphamide. Therefore, immunomodulator agents and irradiation alter the chronic effects of morphine and the opiate withdrawal symptoms. The investigation herein was initiated to study the effects of immunomodulator agents and the whole body irradiation on the acute effects of morphine as measured by analgesia in the tail immersion procedure.

Ten groups of male Sprague-Dawley rats (10/group) weighing 180-200 grams were treated as follows: 1) control saline, 2) Morphine (5 mg/kg ip) group, 3) α-interferon (150 units/gr/body weight); 4) 15 mg/kg cyclosporine A ip group, 5) 500 rads total irradiation, 6) three days food abstinence, 7) interferon and morphine, 8) cyclosporine A and morphine, 9) irradiation and morphine and 10) morphine in fasted animals. The reaction time observed in saline, α-IFN, cyclosporine A, irradiation and starvation (groups 1, 3, 4, 5, and 6) were identical. Groups 7, 8 and 10 (α-IFN and morphine as well as cyclosporine A and morphine) were significantly suppressed and identical to group 2, i.e. to morphine alone. In group 9, irradiation prior to morphine treatment, the analgesic effects of morphine were prevented as assessed in the tail immersion test. In conclusion, immunomodulator agents known to modify the chronic effects of morphine (opiate addiction) failed to modify the acute effects (analgesia) of the drugs. However, ablation of the immune system by whole body irradiation revealed involvement of the immune system in the analgesic effects of morphine. Therefore, the interaction of the immune system in morphine induced analgesia involves mechanisms exclusive those acted on by α-interferon, cyclosporine A, and cyclophosphamide.

351.15 EVIDENCE THAT OPIATE ADDICTION INVOLVES AN IMMUNE RESPONSE. N.E. Kletzly*, P.M. Dougherty*, J. Aronowski*, N.R. Pellis* and N. Dafny (SPON: D.A. Redburn). Department of Neurobiology and Anatomy, UTHMS, Department of Surgery and Organ Transplantation, UTHMS.

We previously reported that selective ablation of the immue system by exposure to ionizing radiation prior to and after chronic morphine treatment (pellet implantation) modified the abstinence syndrome elicited by naloxone. These observations suggest that the immune system is involved in morphine dependence and withdrawal phenomena. The present study was initiated to further assess the role of the immune system in the withdrawal from opiate addiction by selective ablation of immunocompetent cells prior to administration of morphine. Two groups of in-bred male Lewis rats (N=4) were irradiated with $^{137}Cs$ for 0.93 minutes for a total degree of 500 rads. One group received no further treatment. In the other group, the immune system was reconstituted by adoptive transfer of spleen and lymph node cells from two normal donors to one recipient. A 75 mg pellet of morphine base was subcutaneously implanted into each rat and into animals in an additional non-irradiated group (N=12). Seventy-two hours later naloxone (1 mg/kg) was administered intraperitoneally to induce the abstinence syndrome and thereby assess the degree of morphine dependence. Seven behavioral measurements were scored: Wet dog shakes, teeth chatterings, formed stools, diarrhea, hyperactivity, exploratory behavior, and scream-to-touch. Experimental groups were compared to controls implanted with placebo pellets and challenged with saline. Results showed that irradiation suppressed withdrawal in subjects that the immune system was not reconstituted. Statistical evaluation revealed no significant difference between the non-irradiated, normal placebo controls and the irradiated rats given morphine. Furthermore, there was no significant statistical difference among morphine addicted rats whether normal or irradiated and adoptively reconstituted with immunocompetent cells. In conclusion, the present study demonstrated that 1) irradiation suppresses the opiate withdrawal syndrome, and 2) reconstitution of the immune system after whole body irradiation of 500 rads restores the classical naloxone precipitated withdrawal in morphine addicted animals. These observations are evidence that the immune system participates in opiate addiction.

351.16 SPINAL CORD BLOOD FLOW FOLLOWING TRAUMA: EFFECTS OF NALOXONE. G.E. Hollinden* and B.T. Stokes. (SPON: W.E. Hunt) Dept of Physiology, The Ohio State Univ. Sch. of Med., Columbus, OH 43210

Spinal ischemia following spinal cord injury has been associated with distinct pathological alterations in the spinal microenvironment. The resulting lack of molecular oxygen, incipient hypocalcia, and the cascade of rapidly occuring neuropathological events would all augur for a reversal of the ischemic insult. It has been suggested that one of the beneficial effects of the opiate antagonist naloxone might be to prevent this ischemic insult by maintaining spinal cord blood flow at control levels subsequent to injury. We have also reported a reversal of the dramatic injury-induced extracellular hypocalcia after such high dose naloxone regimens. Because of certain discrepancies in previous reports relating spinal blood flow to naloxone therapy and the appearance of newer more quantitative blood flow techniques, we have monitored blood flow in spinal gray and white matter after impact injury and naloxone intervention.

Spinal cord blood flow was measured using the diffusable tracers technique developed by Kety (1960) as modified by Zivin (1983). Random source mongrel dogs were anesthetized and cannulated. A monofilament ligature is placed around the aorta at the branching of the common carotid and exteriorized. The chest is then closed and normal respiratory and blood gas parameters reestablished. A laminectomy is performed and the animal is suspended from a rigid spinal frame and traumatized (400 gm-cm; Allen Drop Technique). Naloxone hydrochloride (10 mg/kg) is administered twenty minutes post-trauma according to our established protocol. At the measurement time, approximately 10 uCi/kg are injected in a IV bolus and blood samples are collected at five second intervals from a branch of the caudal auricular artery over a two minute period. At the end of the two minute period, the aorta is severed with the ligature rapidly terminating blood flow. The spinal cord is quickly removed, frozen and sectioned into gray and white samples. The blood and tissue samples are then processed and counted. The results at sampling times of 30 min., 1.0 hr., and 2.0 hrs. post-trauma were as follows (all flows are expressed as ml/100g/min):

| | 30 MIN. | | 1.0 HR | | 2.0HR | |
|---|---|---|---|---|---|---|
| | Gray | White | Gray | White | Gray | White |
| CONTROL: | 31.5±2 | 14.4±2 | 30.9±3 | 15.7±1 | 48.1±5 | 15.9±.5 |
| TRAUMA: | 10.2±3 | 9.4±1 | 11.1±2 | 14.5±1 | | |
| NALOXONE: | 43.0±3 | 16.1±1 | 33.5±3 | 10.6±1 | 36.4±3 | 7.7±.3 |
| NAL/TRA: | 9.3±5 | 11.3±2 | 13.1±2 | 11.4±2 | 13.1±5 | 12.1±2 |

After trauma, the blood flow drops significantly and remains depressed for the entire period studied. Naloxone has little effect on blood flow by itself and after trauma. We conclude, therefore, that naloxone must exert its effect on neurologic improvement and resolution of extracellular hypocalcia by mechanisms other than a prevention of the ischemic insult. (Supported by USPHS NS-10165).

351.17 AN INTERACTION OF THE IMMUNE SYSTEM AND OPIATE DEPENDENCE: ADOPTIVE TRANSFER OF CYCLOSPORINE'S ATTENUATING EFFECT UPON THE WITHDRAWAL SYNDROME. P. Dougherty,* J. Aronowski,* D. Drath* and N. Dafny (SPON: R. Wiggins). Department of Neurobiology and Anatomy, UTHMS, Department of Surgery and Organ Transplantation, UTHMS.

A detailed mechanism for the development of opiate dependence and the expression of the withdrawal syndrome associated with the drug's interruption has not yet been fully elucidated despite many theories which attempt to explain these phenomena. One theory, suggesting immunologic involvement in opiate dependence, has gained additional support recently by previous studies from our group which have demonstrated an attenuation of the withdrawal syndrome by various immune modulating agents such as cortisol, α-interferon, cyclophosphamide, and cyclosporine-A. The present study was undertaken to further define the mode whereby cyclosporine exerts this effect and to determine if this drug induces a soluble or transferable factor that modifies the development of opiate dependence.

Fischer 344 male rats were divided into 4 basic groups, each consisting of 16 animals. The separate groups were treated with either a placebo regimen or a drug regimen consisting of either cyclosporine, morphine sulfate, or a combination of cyclosporine and morphine, for a time period of three days, the period of continuous exposure necessary to establish a state of dependence. Each group was then divided into two identical groups, half of which were used for behavioral studies and the other half sacrificed and harvested for their spleen populations. Spleen cells were then adoptively transferred to 4 additional recipient groups (N=8) which had undergone prior treatment with placebo regimes or drug regimens. Recipient animals were then assessed for opiate dependence via naloxone administration and scored for 10 common behavioral signs of antagonist-precipitated withdrawal. Results were compared to scores from behavioral animals which received only the various drug treatments followed by naloxone. The results we obtained demonstrated that only splenic cells from cyclosporine treated groups were able to attenuate the withdrawal syndrome. This attenuation was similar to that observed in non adoptively transferred rats receiving cyclosporine and morphine combinations as described previously. In conclusion, the present study demonstrates that the effect of withdrawal attenuation induced by cyclosporine can be transferred from one animal to another by immune components. This observation further suggests an involvement of the immune system in the development of opiate addiction.

351.18 ANALYSIS OF REGIONAL BRAIN UPTAKE AND ACCUMULATION OF MORPHINE BY MASS FRAGMENTOGRAPHY. G.N. Fuller, S.-N. Lin*, R.M. Capriola*, R.C. Wiggins and N. Dafny. Dept. of Neurobiology and Anatomy and The Analytical Chemistry Center, The Univ. of Texas Med. Sch. at Houston, Houston, Tx. 77025.

The regional distribution of morphine has been investigated by numerous laboratories under a number of different experimental conditions, with prominent variables including route of administration, dosage, species, and morphine detection method. However, the regional uptake and accumulation of morphine following an incremental dosage regimen (commonly employed in neurophysiological experimentation) has never been examined. In the present study, morphine distribution was measured in eight brain areas (cerebral cortex, hippocampus, striatum, midbrain, hypothalamus, thalamus, medulla and cerebellum) following with single dose or cumulative doses. For single dose studies, six groups of four animals each received a single, i.p. injection of either phosphate buffered saline, or of morphine sulfate at a dose of 0.5, 1.0, 5.0, 10.0 or 30.0 mg/kg. All injections were in 1 ml total volume. For cumulative dose studies, animals received incremental doses of morphine sulfate (0.5, 1.0, 5.0, 10.0, 30.0 mg/kg), injected i.p. at intervals of 30 min, with groups of four animals being sacrificed after the 0.5, 1.0, 5.0, 10.0 and 30.0 mg/kg doses. For each experimental group, animals were anesthetized with sodium pentobarbital at 15 min after the last morphine injection and quickly perfused for 1 min with 10% formalin solution to clear blood from the brain. Animals were decapitated, the brains dissected into eight regions, and the regions massed on a Mettler PN 1210 analytical balance and frozen in air tight glass vials at -50°C. Regional morphine levels were subsequently determined by gas chromatography-mass spectrometry with chemical ionization detection. Following single dose administration, all brain regions show similar patterns of uptake with increasing dose, with the notable exception of the hypothalamus which demonstrated a linear uptake and the highest concentrations of morphine for any given dose. Following cumulative dose administration, regional morphine uptake profiles show three distinct patterns. Hippocampus, cerebellum, medulla and cerebral cortex show a linear uptake pattern, whereas thalamus, midbrain and striatum show a non-linear profile. As also seen with single dose administration, the hypothalamus is distinctive in its markedly higher morphine concentrations at all dosage levels. The present experiments thus provide evidence for dose-dependent differential uptake and accumulation of morphine in specific regions of rat brain following incremental morphine administration.

352.1 EFFECTS OF L-TYROSINE AND OTHER AMINO ACIDS ON THE TYRAMINE-INDUCED CHRONOTROPIC RESPONSE IN THE ISOLATED RAT HEART. J.M.B. Pinto* and T.J. Maher. Department of Pharmacology, Massachusetts College of Pharmacy, Boston, MA 02115.

The synthesis of catecholamines from the precursor amino acid L-tyrosine (TYR) is regulated by the rate-limiting enzyme tyrosine hydroxylase (TH). Under resting conditions, the activity of TH is dependent upon the availability of the tetrahydrobiopterin cofactor and not the availability of its substrate TYR. When noradrenergic neurons are activated (i.e., firing-frequently), TH is phosphorylated via a protein kinase and the availability of the cofactor no longer limits TH activity, while the concentration of TYR is now the limiting factor. The precursor-dependence phenomenon has been demonstrated in situations where neurons are activated such as hypertension, hypotension, tail-shock stress and electrically-stimulated striatal slices. As expected, when neurons are at rest, no increase in catecholamine synthesis is seen when TYR concentrations are increased. To determine if TYR is capable of enhancing the synthesis of catecholamines in activated neurons in the heart, we compared the tyramine-induced increase in chronotropy in the isolated rat heart perfused with or without TYR.

Isolated hearts were perfused with Chenoweth-Koelle solution according to the Langendorff procedure and positive chronotropic responses evoked with 350 µg tyramine at 10 min. intervals for 60 min. The addition of 50 µM TYR into the perfusion solution did not alter the basal heart rate when compared to control. Tachyphylaxis to tyramine always occurred after the 1st or 2nd injection in control-perfused hearts, while the inclusion of TYR prevented tachyphylaxis for the 60 min. period (TYR-perfused hearts responded to tyramine for up to 8 hrs.). Norepinephrine, a direct agonist, produced a chronotropic response in control-perfused tachyphylactic hearts suggesting that the tyramine-induced unresponsiveness was due to a depletion of stored catecholamines. The inclusion of equimolar concentrations of the L-amino acids: phenylalanine, valine, serine, glutamic acid, aspartic acid, arginine and lysine, or the D-amino acids: tyrosine and tryptophan failed to prevent the onset of tachyphylaxis indicating that a nonspecific effect of amino acids was not responsible for our observations. 3-Iodo-L-tyrosine and alpha-methyl-para-L-tyrosine prevented the TYR-induced response indicating the requirement for catecholamine synthesis. d,l-Propranolol also blocked the TYR-induced response, most likely at the postsynaptic receptor.

We have shown that a peripheral organ in vitro, the isolated rat heart, is dependent upon TYR availability for catecholamine synthesis when neurons fire frequently.

352.2 EFFECT OF CHRONIC COLD ON TYROSINE HYDROXYLASE AND PHENYLETHANOLAMINE-N-METHYLTRANSFERASE mRNAS IN RAT ADRENAL GLAND. M.K. Stachowiak, R. Sebbane*, E.M. Stricker, M.J.Zigmond, and B.B. Kaplan. Depts. of Psychiatry, Psychology, and Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260.

Adaptation of animals to chronic cold involves increased secretion of adrenal catecholamines, which is sustained by increased activity of tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine biosynthesis (Thoenen, Nature 228:861, 1970; Fluharty et al., J. Pharm. Exptl. Ther. 233:32, 1985). The activity of phenylethanolamine-N-methyltransferase (PNMT) also increases slightly during prolonged exposure to cold (Kvetnansky et al., Am. J. Physiol. 220: 928, 1971). Cold-induced increases in the TH activity were found to reflect an increased rate of enzyme synthesis (Chuang & Costa, Proc. Natl. Acad. Sci. 71:4570, 1974). The purpose of this study is to investigate further the mechanism of TH and PNMT induction in cold-adapted rats by examining levels of TH and PNMT mRNAs. Sprague-Dawley male rats with clipped fur were placed in a 5°C chamber for 1 week. Control rats were kept in a temperature controlled environment at 22°C. Total RNA was isolated from adrenal medulla pooled from 10 rats. Estimates of the relative amounts of the TH and PNMT RNAs were obtained by RNA dot-blot hybridization analysis using cloned TH and PNMT cDNAs as hybridization probes (Lewis et al., J. Biol. Chem. 258:14632, 1983; Kaplan et al. In "Gene Expression in Brain," C. Zomzely-Neurath and W.A. Walker, Eds., Wiley Interscience, New York, NY, 1984, pp. 1-22). Specificity of the hybridization reactions was verified by hybridization to total RNA isolated from the rat caudato-putamen, a brain region which apparently does not contain catecholaminergic cell bodies. After 7 days of cold exposure, we observed a 4.8-fold increase in the amount of adrenomedullary RNA which hybridized to TH cDNA. Similar results were obtained by northern analysis using adrenal poly(A+)RNA. The amount of TH cDNA which hybridized to TH RNA had increased 4-fold, demonstrating that the relative abundance of TH mRNA in the cold-exposed rats had increased considerably. The cold-induced increase in adrenal TH mRNA appears rather specific. Results of dot-blot hybridization experiments and northern analysis have shown that the relative abundance of adrenomedullary PNMT mRNA increases by only 20-60% in rats subjected to cold stress. The fact that the changes found in our experiments are similar in magnitude to the reported effects of cold on TH and PNMT activity is consistent with the hypothesis that the changes in enzyme activities are mediated by alterations in the corresponding mRNAs levels. Further investigation will be necessary to determine whether cold-induced changes in the abundance of TH mRNA results from an enhanced rate of transcription, alterations in RNA processing or nuclear-cytoplasmic transport, or decreased rates of mRNA degradation.

352.3 REGULATION OF TYROSINE HYDROXYLATION IN STRIATAL AND PREFRONTAL CORTICAL BRAIN SLICES. M. E. Wolf and R. H. Roth, Yale University School of Medicine, Depts. of Pharmacology and Psychiatry, New Haven, CT 06510.

A number of in vivo biochemical studies have suggested that DA neurons innervating the prefrontal cortex lack synthesis modulating DA autoreceptors. However, apomorphine has been reported (Fadda et al., Brain Res., 1984) to inhibit prefrontal DOPA accumulation in rats pretreated with a decarboxylase inhibitor. Recent work in our lab suggests that this effect is linked to the ability of agonists to modulate intraneuronal levels of DA thereby indirectly regulating the level of feedback inhibition to which tyrosine hydroxylase is subjected. It has been proposed that DA agonists act at release modulating autoreceptors to inhibit ongoing DA release and thereby slow the rapid decline in endogenous DA levels which occurs in prefrontal DA terminals following decarboxylase inhibition. In contrast, nigrostriatal DA terminals appear to possess autoreceptors which modulate tyrosine hydroxylation directly, independent of effects on intraneuronal DA levels. Brain slices provide a potentially useful model system in which to investigate this hypothesis, since 1) there is presumably less impulse flow-dependent DA release in slices, making it possible to assess the effects of DA agonists and antagonists on synthesis modulating autoreceptors without the confound of drug-induced changes in release and therefore in intraneuronal DA levels 2) one can isolate drug effects on the nerve terminal.

Addition of the DOPA decarboxylase inhibitor NSD-1015 (200uM) to striatal or prefrontal cortical brain slices resulted in an accumulation of DOPA that was linear for at least 30 min. DOPA accumulation was stimulated in both regions in the presence of 30mM potassium. The DA antagonist l-sulpiride enhanced $K^+$-stimulated DOPA accumulation in striatal slices, presumably by preventing the activation of synthesis modulating autoreceptors by endogenous DA. Sulpiride had no effect on $K^+$-stimulated DOPA accumulation in prefrontal cortical slices. The non-catechol DA agonist EMD 23 448 produced a dose-dependent inhibition of both basal and $K^+$-stimulated DOPA accumulation in striatal slices. In contrast, concentrations of EMD 23 448 which resulted in greater than 60% inhibition of basal DOPA accumulation in striatal slices had no effect on basal prefrontal DOPA accumulation. However EMD 23 448 did reverse the $K^+$-stimulated enhancement of DOPA accumulation in prefrontal slices. Since $K^+$-stimulated synthesis is presumably associated with greater DA release than is basal synthesis, the effect of EMD on $K^+$-stimulated DOPA accumulation may involve activation of release modulating autoreceptors. These in vitro findings are therefore consistent with the proposed lack of synthesis modulating autoreceptors on mesoprefrontal nerve terminals. (Supported in part by USPHS Grant MH 14092 and the State of Connecticut)

352.4 PHORBOL ESTER ACTIVATES TYROSINE HYDROXYLASE IN THE PERFUSED RAT ADRENAL GLAND. J. P. Mitchell* and P. R. Vulliet (SPON: G. Frye). Dept. of Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX 77843.

The isolated perfused rat adrenal preparation (Wakade, A. R., J. Physiol., 313: 463, 1981) was used to study the regulation of tyrosine hydroxylase (TH) by acetylcholine and by the phorbol ester 4 -phorbol 12 -myristate 13 -acetate (PMA), a potent activator of protein kinase C. The left adrenal gland of anesthetized (pentobarbital, 60 mg/kg, i.p.) male Sprague-Dawley rats (200-300 g) was perfused by inserting a PE 10 catheter into the adrenal vein, ligating all side branches of the vein, and making a small incision through the connective tissue capsule to allow perfusate to escape. The adrenal was perfused with oxygenated Krebs-bicarbonate buffer at a rate of 0.36 ml/min and maintained at 37°C throughout the experiment. In some experiments, the perfusate was collected and analysed for epinephrine and norepinephrine content by HPLC with electrochemical detection. Inclusion of acetylcholine in the perfusion buffer resulted in a large increase in catecholamine release and a significant increase in TH activity. This increase in activity resulted primarily from a decrease in the Km for the cofactor 6-methyl-5,6,7,8-tetrahydropterine ($MPH_4$). The acetylcholine induced activation of TH and release of catecholamines could be blocked by a combination of hexamethonium and atropine, nicotinic and muscarinic cholinergic antagonists, respectively. Perfusion of the adrenal by PMA also resulted in a significant increase in TH activity that resulted from a reduction in the Km of TH for $MPH_4$. These findings suggest that protein kinase C may be involved in the regulation of TH in vivo.

352.5 ACTIVATION OF TYROSINE HYDROXYLASE IN THE RAT SUPERIOR CERVICAL GANGLION BY PEPTIDES OF THE GLUCAGON-SECRETIN FAMILY. M.A. Schwarzschild,* J. Rivier,* W.W. Vale, N.Y. Ip, and R.E. Zigmond. Dept. of Pharmacology, Harvard Med. School, Boston, MA 02115 and Peptide Biology Laboratory, The Salk Institute, San Diego, CA 92138

Preganglionic nerve stimulation has been shown to increase the rate of dopa synthesis measured in intact rat superior cervical ganglia maintained in vitro in the presence of the dopa decarboxylase inhibitor brocresine. This effect is only partially blocked by nicotinic and muscarinic antagonists, suggesting the involvement of a non-cholinergic preganglionic neurotransmitter (Ip et al., PNAS 80:2081, 1983). It was subsequently found that three peptides of the glucagon-secretin family--vasoactive intestinal peptide (VIP), secretin, and PHI also increase dopa synthesis in the ganglion by 4-, 3-, and 2-fold respectively at a concentration of 10 μM . Other peptides in this family, namely glucagon, gastric inhibitory peptide and human growth hormone-releasing factor (hGRF), and many unrelated peptides have no effect when tested under the same conditions (Ip et al., Peptides 5:309, 1984).

To determine the mechanism of the peptidergic stimulation of dopa synthesis, ganglia were incubated with various peptides at 37°C for 30 min without brocresine and then homogenized. The tyrosine hydroxylase (TH) activity of the homogenates was assayed at pH 7.0 in the presence of a subsaturating concentration of pterin cofactor (30 μM 6-methyltetrahydropterin). VIP (10 μM), secretin (10 μM), and PHI (30 μM) increased TH activity by 4-, 3-, and 3-fold respectively, while glucagon (10 μM) had no effect. Further experiments with VIP and secretin demonstrated that these peptides also shifted the pH optimum of ganglionic TH from 5.8 to 6.4. These data suggest that the peptidergic stimulation of dopa synthesis in intact ganglia results from an activation of TH.

Recently GRF from rat hypothalamus (rGRF) has been isolated and sequenced and found to be a member of the glucagon-secretin family. rGRF has 67% sequence homology with hGRF. rGRF (30 μM) increased the rate of dopa synthesis in intact ganglia by 3-fold and increased TH activity in ganglion homogenates by 2.5-fold. rGRF(1-29)$NH_2$ (10 μM) also increased dopa synthesis by 2-fold, while hGRF(1-29)$NH_2$ had no effect. Since the N-terminal residue of hGRF is tyrosine while that of rGRF is histidine, [$Tyr^1$]rGRF(1-29)$NH_2$ was tested, and it was found to produce a 1.4-fold increase in dopa synthesis. hGRF was also examined for its ability to antagonize the effect of rGRF. Addition of hGRF(1-40)OH (30 μM) did not increase the rate of dopa synthesis nor did it antagonize the effect of rGRF (10 μM). rGRF and the various rGRF fragments tested also increased ganglionic cAMP, while hGRF and the fragments of hGRF tested did not. These data are consistent with our hypothesis that the peptidergic stimulation of ganglionic TH activity is mediated by an increase in cAMP accumulation (Ip et al., J. Neurosci., In press). Supported by grants NS12651 and MH00162.

352.6 CHRONIC ADMINISTRATION OF METHAMPHETAMINE DEPLETES TYROSINE HYDROXYLASE IMMUNOREACTIVITY IN THE NIGROSTRIATAL AND MESOLIMBIC DOPAMINE SYSTEMS IN THE RAT BRAIN. M.E. Trulson, M.S. Cannon,* T.S. Faegg*[1] and J.D. Raese[2]. Department of Anatomy, College of Medicine, Texas A&M University, College Station, TX 77843, [1]E. Leitz, Inc., Rockleigh, N.J. 07647 and [2]Schizophrenia Research Center, Southwestern Medical School and V.A. Medical Center, Dallas, TX 75216.

Chronic methamphetamine administration (20 mg/kg, i.p., every 12h for 10 consecutive days) produced a large decrease in tyrosine hydroxylase staining axons and terminal boutons in the caudate nucleus, nucleus accumbens, and frontal cortex in rats when examined 60 days following the final methamphetamine injection, as compared to saline-injected control rats. This effect was quantitated using the Leitz Data Acquisition and Display System (DADS), which revealed that there was a 77% decrease in tyrosine hydroxylase positive nerve terminal processes in the caudate nucleus, and a 66% and 62% decrease in tyrosine hydroxylase positive nerve terminal processes in the nucleus accumbens and frontal cortex, respectively. Furthermore, this chronic methamphetamine treatment regimen also produced a large decrease in the number of tyrosine hydroxylase positive staining neuronal parikarya in the pars compacta of the substantia nigra and in the ventral tegmental area. This latter effect was also quantitated using the Leitz DADS system, which revealed a decrease of 72% in tyrosine hydroxylase positive material in the substantia nigra and a 69% decrease in tyrosine hydroxylase positive material in the ventral tegmental area. Despite these large depletions of brain dopamine, the rats appeared relatively normal upon gross examination 60 days following termination of methamphetamine treatment. These data demonstrate that chronic administration of methamphetamine produces a long term loss of tyrosine hydroxylase enzyme in both the cell bodies of the substantia nigra and the ventral tegmental area and the nerve terminals in the caudate nucleus, nucleus accumbens and frontal cortex. Whether these effects are due to the degeneration of neurons or some metabolic or biochemical effect is currently under investigation.

352.7 TYROSINE HYDROXYLASE ACTIVITY IN POST-MORTEM SAMPLES OF HUMAN PUTAMEN AND CAUDATE NUCLEUS. D.L.Sparks and J.T. Slevin.Veterans Administration Medical Center and the Depts. of Neurology and Pharmacology and the Sanders-Brown Research Center on Aging, University of Kentucky, Lexington, Kentucky 40536

Several neurological disorders, e.g. Parkinson's Disease, include an alteration of dopaminergic synaptic activity in basal ganglia. Tyrosine hydroxylase (TH), which catalyzes the conversion of tyrosine to dopa, plays a central role in the modulation of catecholamine biosynthesis. TH activity is the rate-limiting step in catecholamine synthesis and is subject to end-product inhibition. In order to study TH activity in human post-mortem tissue, it is important to know the effect of several parameters (e.g. age of the subject, time from death to tissue analysis, ambient temperature) on the enzyme's activity. To insure consistency and facilitate the handling of large numbers of samples, it is important to have a simple, quick and easily-reproduced method.

We have recently developed a method for determining TH activity by liquid chromatography with electrochemical detection (LCEC) which requires no elaborate preparation, pre-column "clean-up", or recovery studies. Data relating TH activity in human caudate nucleus and putamen to the post-mortem interval (from cardiorespiratory death to sample analysis) and to age will be presented. As in rat (Black, I., Arch.Neurol. 32:47, 1975), post-mortem TH activity in human putamen and caudate nucleus is stable for a short time (< 24 hours), after which activity decreases precipitously. Comparisons between our method and a widely used radiometric assay (Coyle, J.T., Biochem.Pharmacol. 21:1935, 1972) and LCEC assay (Lin, P.Y.T. et al., J.LiquidChrom. 7:509, 1984) are made.

Supported in part by the Veterans Administration Research Service, the NIH (TIDA 00732 - JTS), Univ. of Kentucky PSP Fellowship (DLS) and ADRDA (DLS).

352.8 GENETIC CORRELATION ANALYSIS OF HYPOTHALAMIC AND MIDBRAIN TYROSINE HYDROXYLASE ACTIVITY. L.R. Murthy,* I. Sziraki,* A. Lajtha and Cs.Vadasz*). (SPON: S. P. Bagchi). Lab. of Neuro-behavior Genetics, Neurochemistry Div., Nathan Kline Institute, Orangeburg, NY 10962.

Differences in tyrosine hydroxylase (TH) activity in hypothalamus (HT) and midbrain (substantia nigra - A-10 region, SN) were reported between male mice of BALB/cJ(C), and CBA/J strains (Ross et al. Nature, 264:654, 1976; Baker et al. J. Neurosci., 3:832,1983). Our studies using reciprocal F1 hybrids indicated significant genetic additive effects in SN ($p<0.05$), significant additive and maternal effects in hypothalamus (Vadasz et al. 1985, in press).

Comparison of these two strains and their hybrids showed that higher levels of TH activity in SN (TH/SN) and hypothalamus (TH/HT) were associated with the BALB/cJ strain, indicating the possibility of common genetic control.

To study further the genetic control over central dopamine systems, strains with high (BALB/cJ) and low (CXBI/J)(I) TH/SN were chosen and each were crossed with a strain of intermediate TH/SN (C57BL/6ByJ) (B6).

Intercrossing the (B6XI)F1 and (B6XC)F1 generations, produced replicated (B6XI)F2 and (B6XC)F2 segregating populations, thereby providing an opportunity to estimate the environmental and genetic sources of the phenotypic correlations between TH/HT and TH/SN and also between TH/HT and TH activity in corpus striatum (TH/CS). The two types of crosses (B6XI) and (B6XC) yielded different results:

a) significant differences were found in TH/HT between B6, C and (B6XC)F1 with high heritability of TH/HT for (B6XC)$F_2$ ($h^2_b = 0.66$);
b) no significant differences were found in TH/HT between B6,I and (B6XI)F1 with low or negligible heritability of TH/HT for (B6XI)$F_2$ ($h^2_b=0.01$).

Consequently, phenotypic ($r_P$), environmental ($r_E$) and genetic ($r_G$) correlation coefficients were determined only for the (B6XC)F2 generation.

| | $r_P$ | $r_E$ | $r_G$ |
|---|---|---|---|
| TH/HT and TH/SN | 0.25 | -0.096 | 0.45 |
| TH/HT and TH/CS | 0.33 | 0.10 | 0.51 |

Significant differences were reported in TH/SN between $B_6$ and I (Vadasz et al., Brain Res., 234: 1-9, 1982), but not in TH/HT, as measured in this study, suggesting a difference in the genetic control of the hypothalamic and nigrostriatal catecholamine systems. However, the significant genetic correlations found in the present studies, demonstrate that there is also a set of common genes which influence TH activities in all three regions (HT,SN,CS) through pleiotropy or linkage of genes.

352.9 GENETIC CORRELATION ANALYSIS OF THE NIGROSTRIATAL DOPAMINE SYSTEM. I. Sziraki*, L.R. Murthy*, A. Lajtha and Cs. Vadasz*(SPON: R. Squires). Lab. of Neuro-behavior Genetics, Neurochemistry Div., The N. S. Kline Inst. for Psychiatric Research, Orangeburg, NY 10962

In recent experiments with Recombinant Inbred Strains significant and positive between-strain correlation (r=0.82) was found between tyrosine hydroxylase (TH) activities in substantia nigra (SN) -A-10 area and corpus striatum (CS). These results suggested that there is a common set of genes influencing both number of dopamine (DA) neurones in substantia nigra-A-10 region and axonal arborization of nigral DA neurones in corpus striatum (Vadasz et al., Brain Research, 234,1., 1982).

In the present study we investigated the correlation between TH activities in SN-A-10 region and CS further. On the basis of the previous results those strains were selected which had the lowest (CXBI/J) and highest (BALB/cJ) TH activity in SN-A-10 area. These strains were crossed with a strain of intermediate TH activity in SN (C57BL/6ByJ). The resulted (B6I)F1 and (B6C)F1 hybrids were intercrossed within their own generation to produce (B6I)F2-ALPHA, (B6I)F2-BETA, (B6C)F2-ALPHA and (B6C)F2-BETA segregating populations. The analysis of the statistically nontransformed data indicates that in TH activity of SN the B6 strain is dominant (or partially dominant) in both types of crosses (B6XI and B6XC). Phenotypic variances in B6IF2-ALPHA and B6IF2-BETA are smaller than the enviromental variance estimated from the genetically homogenous populations. Heritabilities are high and the estimates of minimum number of genes are low for B6CF2-ALPHA and B6CF2-BETA segregating populations.

Similarly, in the TH activity of CS the B6 strain is dominant (or partially dominant) in both crosses and the range of heritabilities is between 0.20 (B6IF2-BETA) and 0.64 (B6CF2-ALPHA). The estimates of minimum number of genes involved are smaller for the B6CF2 generations than for the B6IF2 generations.

Correlation analysis of the TH activities in SN and CS using more than 200 animals of the progenitor strains, hybrids and F2 generations indicates low and nonsignificant enviromental correlation ($\bar{r}_E$=0.15) and high and significant genetic correlations in the B6CF2 generations (0.71 and 0.82).

These results demonstrate that:

1.) the genetic properties of the B6XI and B6XC type crosses in controlling TH activities of the nigrostriatal system are different

2.) the genetic correlation coefficients, obtained from large segregating populations, are high and significant providing further support to the hypothesis of common genetic control of number of DA neuron cell bodies in SN-A-10 area and axonal arborization of DA neurones in corpus striatum.

352.10 MONOCLONAL ANTIBODIES DIRECTED AGAINST TYROSINE HYDROXYLASE. S.W.Snell*, S.Kwan*, C.W.Abell*, R.M.Denney*, and P.R.Vulliet . Dept. of Vet. Phys. and Pharmacol., Texas A&M Univ., College Station, TX 77843 and Dept. of Human Biological Chemistry and Genetics, The Univ. of Texas Med. Branch, Galveston, TX 77550.

Monoclonal antibodies directed against tyrosine hydroxylase (TH) were prepared by injection of 20 ug of the highly purified rat pheochromocytoma enzyme into mice. Lymphocytes were isolated from the spleen and fused with P3/X63Ag8 myeloma cells using polyethylene glycol. Viable cells secreting IgG were selected using aminopterin and ELISA techniques. Clones secreting antibody directed against TH were screened using secondary immunoprecipitation with S. aureus protein A. Disappearance of TH activity from the supernatant and appearance of the activity in the pellet was demonstrated.

Monoclonal antibodies from clone 2D8 were linked to Sepharose 4B using a cyanogen bromide coupling procedure. The antibody-Sepharose complex was equilibrated with phosphate buffered saline (PBS) (0.20g KCl, 0.20g $KH_2PO_4$, 1.14g $Na_2HPO_4$, 8.00g NaCl per liter) and washed thoroughly with a 0.1% BSA solution. $^{125}$I-TH was run over the Sepharose 4B-IgG column. Following washes with PBS, 2 M sodium bromide, and 4 M potassium thiocyanate, the $^{125}$I counts were eluted off the column with 200 mM glycine/HCl, pH 3.0. TH activity was present in the fractions containing the $^{125}$I label. The TH peak was pooled and run on a SDS-PAGE gel. Two bands appeared: 66,000 daltons (BSA) and 60,000 daltons (TH).

Rat adrenal homogenate was then run over the Sepharose 4B-IgG column. After collecting the appropriate fractions from the 200 mM glycine/HCl pH 3.0 eluate, SDS-PAGE gels were run. Again, only 66,000 and 60,000 dalton bands appeared.

Therefore, these antibodies can be used for extracting TH from adrenergic tissues using immunoaffinity chromatography.

352.11 CYCLIC AMP AND GLUCOCORTICOIDS INCREASE THE ENZYME LEVEL AND RATE OF SYNTHESIS OF TYROSINE HYDROXYLASE AND THE LEVEL OF mRNA FOR TYROSINE HYDROXYLASE IN A RAT PHEOCHROMOCYTOMA CELL LINE. A.W. Tank, L.N. Ham* and P. Curella*. Univ. Colorado Health Sci. Ctr, Denver, CO 80262.

Tyrosine hydroxylase (TH) activity and RNA coding for TH ($mRNA^{TH}$) have been shown to be elevated in rat pheochromocytoma cells treated with either glucocorticoids or 8-bromocyclic AMP (J. Biol. Chem. 258, 14,632 1983). In the present report we characterize further the effects of cyclic AMP and glucocorticoids either alone or in combination on the enzyme levels and rates of synthesis of TH and the levels of $mRNA^{TH}$ in rat pheochromocytoma PC18 cells, a subclone of the rat pheochromocytoma PC12 cell line. In these studies TH activity was measured by the coupled decarboxylase assay (Anal. Biochem. 43, 558 1971). The rate of synthesis of TH was measured by incubating cells in the presence of $^3$H leucine and isolating radiolabeled TH by immunoprecipitation using monospecific antiserum to TH. $mRNA^{TH}$ was measured by hybridization to the cloned cDNA probe pTH.4 (J. Biol. Chem. 258, 14,632 1983).

TH activity in the PC18 cells is increased by treatment with either 8-bromocyclic AMP or glucocorticoids. This increase in activity is due to an increase in enzyme protein, as demonstrated by immunoprecipitation studies. Time course studies demonstrate that the enzyme levels rise slowly to a level 6-7-fold greater than that observed in control cells after 4 days of treatment with either 8-bromocyclic AMP or dexamethasone alone. In contrast, the rate of synthesis of the enzyme increases more rapidly, reaching a level 6-7-fold greater than that observed in control cells after 12 hours of treatment with either inducing agent alone. The level of $mRNA^{TH}$ in the cells increases at approximately the same rate as the rate of synthesis of the enzyme to a level 6-7-fold greater than that observed in control cells after 12 hours of treatment with either inducing agent alone. This data strongly supports the hypothesis that cyclic AMP and glucocorticoids increase the level of $mRNA^{TH}$, which results in an increase in the rate of synthesis of the enzyme in the cells. The slow rate of increase in enzyme levels is due most likely to the relatively long half-life of the enzyme in the PC18 cells (half-life = 25-30 hours).

When PC18 cells are treated simultaneously with both 8-bromocyclic AMP plus dexamethasone, the increases in the enzyme level and rate of synthesis of TH are approximately equal to the sum of the increases in these parameters observed in cells treated with either inducing agent alone. In contrast, the increase in the level of $mRNA^{TH}$ in cells treated with both 8-bromocyclic AMP and dexamethasone is equal to the greatest increase observed in cells treated with either inducing agent alone. There is no additive increase in the levels of $mRNA^{TH}$ in cells treated simultaneously with both inducing agents. This surprising observation suggests that in the presence of elevated levels of both cyclic AMP and glucocorticoids, TH is regulated by both transcriptional and post-transcriptional mechanisms. Supported by USPHS grant NS19749.

352.12 FEEDBACK INHIBITION OF PTERIN BIOSYNTHESIS IN THE ADRENERGIC NEUROBLASTOMA N1E115 BY TETRAHYDROBIOPTERIN. G. Kapatos. Laboratory of Neurochemistry, Center for Cell Biology Sinai Hospital of Detroit, 6767 West Outer Drive, Detroit, MI 48235.

The adrenergic neuroblastoma N1E115 synthesizes both D-erythro-neopterin (Neo) and L-erythro-biopterin (Bio) as determined by sequential reverse-phase and cation-exchange HPLC techniques. Determination of intracellular volume showed the average intracelluar concentration of Bio and Neo to be approximately 115 uM and 5 uM, respectively. Neo was present primarily as the completely oxidized or 7,8-dihydro species. By contrast, over 95% of Bio was found to be 5,6,7,8-tetrahydrobiopterin. The level of either pterin in these cells was not dependent on cell cycle or on the concentration of fetal calf serum. Pterin content was therefore independent of cellular differentiation and the alterations in the specific activity of tyrosine-3-monooxygenase reported to occur in this cell line as cell division subsides. In order to investigate the regulation of pterin biosynthesis the initial precursor, guanosine triphosphate, was labelled with [$^{14}$C]guanosine. The biosynthesis of [$^{14}$C]pterins determined by incubation with [$^{14}$C] guanosine was linear for up to 1 hour. Initial rates of synthesis of [$^{14}$C]Neo and [$^{14}$C]Bio from [$^{14}$C] guanosine were inhibited in a concentration-dependent manner by addition of exogenous (6R)-tetrahydrobiopterin to the culture medium, with an $IC_{50}$ of less than 50 uM. This decrease in synthesis was not the result of either: 1) a decline in the labeling of [$^{14}$C]GTP from [$^{14}$C] guanosine, 2) an increase in [$^{14}$C]pterin degradation or 3) a stimulation of [$^{14}$C]pterin efflux into the culture medium. Inhibition of [$^{14}$C]pterin synthesis was also manifest following incubation with similar concentrations of 6-lactyl-7,8-dihydropterin (sepiapterin), or 7,8-dihydrobiopterin, compounds which were rapidly converted to tetrahydrobiopterin within these cells. Studies with a semi-purified preparation from these cells of the first enzyme in pterin biosynthesis, GTP cyclohydrolase, indicated that these pterins directly inhibit this enzyme and display $IC_{50}$ values ranging between 500 and 800 uM. Similar studies are presently being performed using primary neuronal cultures derived from the embryonic mouse. Preliminary data indicate that neurons in spinal cord cultures synthesize substantial quantities of tetrahydrobiopterin and may be a model system for studying the regulation of pterin biosynthesis in a nontransformed cell type.

352.13 TETRAHYDROPTERIN ANALOGS WHICH REPLACE TETRAHYDROBIOPTERIN AS COFACTORS FOR TYROSINE HYDROXYLASE AND WHICH DO NOT CHANGE AFFINITY FOR TYROSINE. J. F. Reinhard, Jr., G. K. Smith*, E. C. Bigham*, R. C. Morrison, Jr.* and C. A. Nichol*. The Wellcome Research Laboratories, Research Triangle Park, NC 27709.

As part of a research program on the potential of biopterin cofactor replacement therapy to stimulate dopamine synthesis in Parkinson's disease (PD), we have synthesized and tested tetrahydropterins with different physicochemical and pharmacokinetic properties. Dopamine (DA) synthesis requires adequate amounts of the co-substrates tyrosine and oxygen and of tetrahydrobiopterin ($BH_4$) as cofactor. Previous reports have established that $BH_4$ levels are diminished in the cerebrospinal fluid of patients with PD (LeWitt, P. A. *et al.*, *Adv. Neurology*, **40**:459-462, 1984). Other work suggests that the tyrosine substrate may also be limiting (Growdon, J. H. *et al.*, *Life Sci.*, **30**:827-832, 1982). Since previously known synthetic $H_4$pterin cofactors for TH increase the $K_m$ for tyrosine, *in vivo* they may increase the requirement for a potentially limiting substrate. We now report the discovery of a new class of synthetic $H_4$pterin cofactors which do not significantly elevate the tyrosine $K_m$ for TH. Additionally, some of these cofactors appear selective for TH, relative to phenylalanine hydroxylase (PH). The TH was prepared from bovine adrenal medulla by partial trypsin digestion and ammonium sulfate fractionation. The activity of TH was measured by the release of $^3H_2O$ from L-3,5-[$^3$H]-tyrosine. The phenylalanine hydroxylase preparation was purified from rat liver by hydrophobic column chromatography, and assayed by measurement of the tyrosine produced using liquid chromatography with fluorescence detection. The $H_4$pterins differ in the substituents at the C6 position (R). The $K_m$ values are micromolar and the $V_{rel}$ values are percentages of the $V_{max}$ values obtained with $BH_4$.

| R | TH | | | PH | |
|---|---|---|---|---|---|
| | $V_{rel}$ | $K_{m_{ptr}}$ | $K_{m_{tyr}}$ | $V_{rel}$ | $K_{m_{ptr}}$ |
| $BH_4$ | 100 | 107 | 15 | 100 | 6 |
| $CH_3$(6-MPH$_4$) | 154 | 133 | 51 | 238 | 21 |
| 6,6-$(CH_3)_2$ | 119 | 77 | 105 | 39 | 29 |
| $CH_2$-O-Methyl | 126 | 84 | 22 | 138 | 17 |
| $CH_2$-O-Ethyl | 154 | 52 | 23 | 155 | 21 |
| $CH_2$-O-Propyl | 92 | 28 | 22 | 32 | 16 |
| $CH_2$-O-Butyl | 135 | 95 | 25 | 22 | 21 |

As can be seen, all of the substituted $H_4$pterins supported tyrosine and phenylalanine hydroxylation. Affinities for TH approached or exceeded that of the natural cofactor and yielded substrate $K_m$ values which were not radically different from the substrate $K_m$ values obtained when $BH_4$ was used as a cofactor for TH. In contrast, the commonly used 6-MPH$_4$ yielded a substrate $K_m$ some 3-fold higher than the substrate $K_m$ obtained using $BH_4$ and the substrate $K_m$ obtained when 6,6-dimethyl-$H_4$pterin was used as cofactor was nearly 10-fold higher than that produced by $BH_4$. Additionally, the 6-butyloxymethyl-$H_4$pterin yielded higher $V_{max}$ values for TH than for PH, and thus appeared selective for TH. The *in vivo* effects of these new pterins should be greater than that of previously synthesized pterins since they are less likely to cause tyrosine to become limiting.

## CATECHOLAMINES: SYNTHESIS AND METABOLISM

353.1 A NEW TECHNIQUE FOR NAFION COATING CARBON FIBER ELECTRODES FOR IN VIVO ELECTROCHEMISTRY. M.P. Brazell*, B. Moghaddam* and R.N. Adams, Depts. of Physiology and Pharmacology, Univ. of Nottingham Medical School, Nottingham, England, and Dept. of Chemistry, University of Kansas, Lawrence, KS 66045.

A well-recognized problem with in vivo electrochemistry is its lack of compound specificity. Many compounds found in brain such as dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and ascorbic acid (AA) oxidize at very similar potentials. Thus it is not possible to resolve between the current generated by their oxidations. A year ago we reported a major improvement in the selectivity of small graphite epoxy capillary (GEC) electrodes used for in vivo electrochemistry (Gerhardt, G.A. et al., Brain Res., 290: 390, 1984). These electrodes were dip-coated with Nafion, a perfluorosulfonated polymer. The anionic Nafion is highly permeable to cations such as DA and norepinephrine (NE), but impermeable to anions such as AA and DOPAC. For the large (200-300 µm tip dia.) GEC electrodes, one or two simple dippings in Nafion and air-drying between dips produces electrodes that are sensitive to neurotransmitters such as DA and insensitive to their metabolites and AA. For the smaller carbon fiber electrodes, because of their smooth surface, such a dipping process gives poor Nafion coatings and thus electrodes without the desired characteristics. We wish to report a simple method for coating the small carbon fiber electrodes (40 µm o.d.). To produce well-coated fiber electrodes, they are washed with double-distilled water and dried gently for 90 sec with a hair-dryer. The electrodes are then horizontally immersed in a Nafion drop placed on a platimum wire. A potential of +0.1 V is applied to the electrode for 5 sec. The electrode is then gently blow-dried for 10 sec. Two further horizontal dips are usually required to produce a sufficient coat; however, these are done without a potential being applied to the electrode.

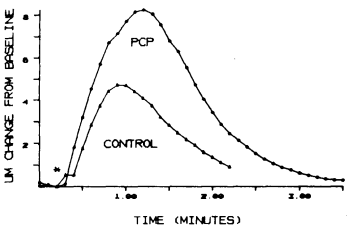
The responses of coated and uncoated fiber electrodes have been tested and compared in vivo by pressure ejection of various mixtures of anionic and cationic oxidizable species next to the electrodes. The same "electroplating" procedure has been successfully used to Nafion-coat GEC electrodes (supported by the British Medical Research Council and National Institutes of Health).

353.2 **PRESYNAPTIC ACTION OF PHENCYCLIDINE (PCP) IN THE RAT STRIATUM DEFINED USING IN VIVO ELECTROCHEMICAL METHODS.** G. Gerhardt[1] and G. Rose[1,2]. [1]Dept. of [2]Pharmacol., University of Colorado Health Sciences Center and [2]Medical Research, VAMC, Denver, CO 80262.

Local application of PCP potentiates catecholaminergic transmission in the CNS. While the mechanism by which this occurs is still unclear, current work in our laboratory suggests that this action of PCP is effected either by eliciting transmitter release, or preventing its reuptake, by presynaptic elements. In an effort to distinguish between these two possibilities, we have used in vivo electrochemical recording methods to examine the effects of PCP upon the dopamine-containing afferents in rat striatum.

Animals were anesthetized with chloral hydrate; chronoamperometric recordings were made using Nafion-coated graphite epoxy capillary (GEC) electrodes, which are very selective for the monoamine neurotransmitters (Gerhardt et al., Brain Res., 290; 390). Local application of PCP and other agents was accomplished using single- or multi-barrel glass micropipettes which were fixed to the GEC electrodes with wax; drugs were applied using micro pressure-ejection.

Application of PCP (barrel concentrations $10^{-4}$ M and $10^{-5}$ M) did not cause an increase in baseline electrochemical signals recorded from the striatum. However, marked increases in both the magnitude (50%) and timecourse (30%) of monoamine releases evoked by local application of $K^+$ were observed if PCP was applied preceeding the $K^+$ bolus (see figure below). Interestingly, excessive application of PCP, or the use of $10^{-3}$ M barrel concentration of this compound, greatly attenuated or abolished $K^+$-evoked releases of monoamines. PCP-induced alterations in the magnitude and temporal dynamics of $K^+$-evoked signals were mimicked by local application of the putative dopamine reuptake blocker nomifensine. Magnitudes and timecourses of electrochemical signals recorded as a consequence of direct local application of dopamine into the striatum from another pipette barrel were also augmented following local PCP administration.




Taken together, these data support the idea that the primary mechanism by which PCP augments catecholaminergic transmission is via interference with neurotransmitter reuptake processes. (Supported by UPSHS grants DA 02429, DA 07043 and the VA Medical Research Service.)

353.3 HABENULAR STIMULATION INCREASES DOPAMINE UTILIZATION AND LOCOMOTOR ACTIVITY. G. R. Christoph*, K. S. Wilcox*, S.-Y. Tam, B. A. Burkhart*, and R. H. Roth. (Spon: J. S. Schwaber) Central Research & Development Department, E. I. du Pont de Nemours & Company, Wilmington, DE and Department of Pharmacology, Yale University, New Haven, CT.

Neurons of the habenula project to the ventral tegmentum where A10 dopamine (DA)-containing neurons are located. The function of this projection was studied by stimulating the habenula and then measuring DA and DOPAC concentrations in the prefrontal cortex (PF), nucleus accumbens (NA), and striatum (ST). Two weeks after unilateral implantation of bipolar electrodes, rats (N=14) received habenular stimulation (250 μA, 250 μsec, 20 Hz) for 30 min in a locomotor activity test chamber. The implanted control group (N=13) was not stimulated. The rats were immediately decapitated and the brains were removed and dissected over ice. HPLC electrochemical detection methods were used to determine DA and DOPAC concentration. Electrode locations in the habenula were verified histologically. In PF samples stimulation caused a significant increase in DOPAC (54%, $P<.03$) compared to control. Stimulation decreased DA in NA by 26% ($p<.001$). ST values were unaffected. Locomotor activity counts were markedly increased by stimulation (366%, $p<.001$). Wet dog shakes were often noted in the stimulated group and never occurred in control rats. As a control for current spread to thalamic nuclei, stimulation of medial dorsal thalamus in another group of rats (N=6) had no effect on the behavioral measures, and unlike habenular stimulation, it only increased DA in PF. The results indicate that habenular stimulation affects mesolimbic and mesocortical dopaminergic function and, in particular, increases dopamine utilization in cortex. Interestingly, the neurochemical and behavioral effects of habenular stimulation are similar to those of infusing substance P analogs into the VTA. Since the medial habenula may be a source of substance P-like compounds in the region of the VTA, we suggest that activation of this peptidergic system is responsible for our results.

353.4 COMPARISON OF DOPAMINERGIC NEURONAL ACTIVITIES IN THE HYPOTHALAMUS AND POSTERIOR PITUITARY GLAND OF MALE AND FEMALE RATS: EFFECTS OF CASTRATION. J.W. Gunnet*, K.J. Lookingland and K.E. Moore. Dept. of Pharmacology/Toxicology, Michigan State Univ., East Lansing, MI 48824.

Sexual differences have been found in the activity of tuberoinfundibular dopaminergic (TIDA) neurons in the rat, with greater activity in the female. The goals of this study were to: 1) compare the activities of TIDA, tuberohypophysial dopaminergic (THDA) and incertohypothalamic dopaminergic (IHDA) neurons in Long-Evans male and female rats and, 2) to determine the effects of castration upon the activity of these 3 dopaminergic systems.

Dopaminergic neuronal activity was estimated by measuring the decline in tissue dopamine (DA) concentrations 0, 30 and 60 minutes after α-methyltyrosine injection (250 mg free base/kg, i.p.) by radioenzymatic assay. DA turnover rates were calculated from DA concentrations in tissue samples collected by means of a modified punch dissection technique. Several brain regions (nucleus accumbens, rostral periventricular nucleus ($A_{14}$), preopticosuprachiasmatic nucleus, medial preoptic nucleus, medial zona incerta ($A_{13}$), dorsomedial nucleus, median eminence) and both intermediate and neural lobes of the pituitary gland were sampled.

As previously reported, the DA activity of TIDA neurons within the median eminence was 2 to 3 times greater in diestrous female rats than intact male rats. The THDA neurons terminating in the intermediate lobe showed no sex differences while those innervating the neural lobe did express a higher DA turnover rate in intact males. Rostral but not caudal IHDA neurons (e.g., neurons within the medial preoptic nucleus versus neurons in the dorsomedial nucleus) exhibited greater activity in the diestrous female versus the intact male. These data demonstrate that the diestrous female when compared to the intact male, has greater DA turnover (i.e., activity) in the TIDA and rostral IHDA systems but lower DA turnover in the THDA neurons of the neural lobe. In the male rat orchidectomy reduced the turnover rate of DA in rostral IHDA neurons and increased the turnover rate of DA in TIDA neurons. Ovariectomy of the female rat decreased TIDA activity and increased the DA turnover in both rostral IHDA neurons and THDA neurons of the neural lobe. These findings indicate that the neurons of the IHDA and THDA systems respond differently to castration in male and female rats and suggest that the hypothalamic DA systems of the female are more responsive to the effects of castration than those of the male. (Supported by USPHS grant NS15911.)

353.5 ANXIOGENIC BETA-CARBOLINE SELECTIVELY INCREASES MONOAMINE METABOLISM IN THE PREFRONTAL CORTEX AND THE HYPOTHALAMUS. S.-Y. Tam, A. Y. Deutch, J.-X. Yang*, M. J. Bannon and R. H. Roth, Departments of Pharmacology and Psychiatry, Yale University School of Medicine, New Haven, Connecticut 06510

We have recently shown that the selective increase in dopamine (DA) metabolism induced in the mesoprefrontal DA neurons by mild footshock stress is modulated by central benzodiazepine (BZ) receptors. Beta-carbolines are specific BZ receptor ligands, some of which have been shown to be anxiogenic in animals and humans. We have examined whether the anxiogenic beta-carboline FG 7142 is able to selectively activate the mesoprefrontal DA neurons in a similar fashion to that induced by stress or conditioned fear.

FG 7142 administered at 5, 10, 15, and 20 mg/kg, i.p., 30 minutes prior to sacrifice, produced a significant dose-dependent increase in DOPAC levels in the prefrontal cortex (PFC). However, DOPAC levels in other mesocortical terminal fields such as the cingulate and piriform cortices were not affected. At 10 mg/kg, DOPAC levels in both the olfactory tubercle and the striatum were significantly decreased, but no change was observed in the nucleus accumbens. The effects of FG 7142 in the PFC were not seen 15 minutes after drug treatment, and disappeared 60 minutes after FG 7142 administration. Moreover, when rats were pretreated with the BZ receptor agonist diazepam or BZ receptor antagonists such as RO15-1788 or CGS 8216, the beta-carboline induced increase in PFC DOPAC levels was significantly antagonized by 75%. At a dose of 15 mg/kg, FG 7142 caused a significant increase in DOPAC levels in the ventral tegmental area (VTA); however, levels of the DA metabolite in the substantia nigra (SN) were significantly decreased. While footshock stress causes a reduction of substance P levels in the VTA, neither substance P nor substance K levels in the VTA or SN were changed 30 minutes after FG 7142 administration. Levels of the norepinephrine metabolites, MHPG and DHPG, were also determined in hypothalamus, cerebral cortex, hippocampus and cerebellum 30 minutes after FG 7142 administration. Significant increases in both MHPG and DHPG levels were observed in the hypothalamus, but not in any other brain region examined. Moreover, the effects of FG 7142 on the hypothalamus were time-dependent and were sustained 60 minutes after drug treatment.

These data demonstrate that the mesoprefrontal DA neurons which are selectively activated by stress, are also selectively activated by the anxiogenic BZ receptor inverse agonist, FG 7142. Since FG 7142 did not appear to activate the substance P input to the VTA, we suggest that the beta-carboline may exert its effects on the mesoprefrontal DA neurons by directly acting on BZ receptors in the VTA. These studies are consistent with multiple types of regulatory mechanisms controlling the function of VTA DA neurons. (Supported by USPHS Grant MH 14092 and the State of Connecticut)

353.6 PHARMACOLOGY AND REGULATION OF MESOAMYGDALOID DOPAMINE NEURONS. C.M. Anderson*, D.L. Knight*, L.E. Elliott*, T.D. Ely* and C.D. Kilts* (Spon.: D. Hernandez). Duke Univ. Med. Ctr., Durham, NC 27710.

The study of dopamine (DA) neuronal innervation of limbic brain structures has been overly focused on the DA-rich nucleus accumbens (NA) and olfactory tubercles (OT). We have previously reported on the significant and heterogeneous DA innervation of the amygdala, and the atypical representation of DA mechanisms thought to be operative in terminal regions of other DA systems (Kilts et al., Neurosci. abst. 10:881, 1984). Whether these differences compared to more conventional DA systems impart a unique pharmacology to the mesoamygdaloid DA neurons, perhaps as a function of unconventional neuronal regulatory mechanisms, was the focus of this investigation.

The effects of drug treatments on biochemical estimates of the functional activity of DA neurons involved determination of (1) the concentration of DOPAC and HVA, (2) DA turnover or utilization following synthesis inhibition and (3) in vivo tyrosine hydroxylase activity estimated by DOPA accumulation following decarboxylase inhibition. All determinations were performed at the level of discrete brain nuclei micropunched from frozen coronal rat brain slices (Palkovits, Brain Res. 59: 449, 1973). DOPA, DA, DOPAC and HVA concentrations were determined by novel on-line trace enrichment HPLC-electrochemical detection methods.

Of the amygdaloid nuclei, the central amygdaloid nucleus (CNA) receives the densest DA innervation. DA neurons innervating the CNA respond to a gammabutyrolactone(GBL)-induced loss of impulse activity with an atypical decrease in DOPA accumulation, an effect enhanced by prior apomorphine (APO) treatment (1 mg/kg, s.c.). In the medial amygdaloid nucleus (MNA), GBL produced an APO-reversible increase in DOPA accumulation similar to that seen in the NA, OT and caudate nucleus (CN). These data suggest that the DA terminals innervating the CNA, like those projecting to the medial prefrontal cortex, lack DA synthesis regulating autoreceptors and thereby differ from neuronal terminations in the NA, OT, CN and MNA. Like the NA, OT and CN the acute administration of haloperidol (1 mg/kg, i.p.) increased all three biochemical estimates of DA neuronal activity in the CNA.

These data further indicate that there are fundamental pharmacological and neuroregulatory differences both within and between limbic structures. An obvious corollary is that the study of DA mechanisms in the NA and OT should not be generalized to DA function in the limbic system when testing the hypothetical involvement of DA in limbic psychopathology. (Supported by NIHM MH-39967.)

353.7 RAPID POST-MORTAL INCREASE IN EXTRACELLULAR CONCENTRATION OF DOPAMINE IN THE RAT BRAIN AS ASSESSED BY INTRA-CRANIAL DIALYSIS. Arnold G. Vulto*, Trevor Sharp and Urban Ungerstedt (SPON: European Neuroscience Association) Department of Pharmacology, Karolinska Institute, P.O.Box 60400, S-104 01 Stockholm, Sweden. *Present address: Rudolf Magnus Institute for Pharmacology, Medical Faculty University of Utrecht,Vondellaan 6, 3521 GD Utrecht,The Netherlands

Measurement of the release of endogenous or preloaded, labelled dopamine (DA) from rat brain slices is commonly used in neuropharmacological studies. Whilst it is known that preloaded DA is preferentially released, it is assumed, that release of endogenous DA in vitro represents the true physiological releasable pool. However, little is known about changes in intraneuronal pools of the endogenous neurotransmitter postmortem. Recently we have shown rapid postmortem increases in the concentration of the DA metabolite 3-methoxytyramine (Vulto et al., 9th IUPHAR congress London, 1984, abstract 1153) which could reflect the release of endogenous DA at death. We have now directly examined this possibility by following changes in the extracellular concentrations of endogenous DA before and after death in the rat brain using a recently developed intracerebral dialysis method in combination with HPLC-ECD (Zetterström et al., J. Neurochem., 41:1769, 1983). Dialysis probes were implanted into the nucl. accumbens of halothane-anaesthetised rats and DA and its metabolite DOPAC were measured in perfusates collected before and after cervical dislocation. Before death typical recoveries in a 20 min perfusate were 0.1-0.2 pmol of dopamine and 25-50 pmol of DOPAC. After death, we sampled every minute. After 2 to 4 min the output of dopamine increased rapidly to more than 1 pmol per min. The dopamine concentration peaked rapidly, 2 to 3 min after the onset of rise,with a gradual decline afterwards. DOPAC showed a small increase in the first minute after death with a subsequent continuous decline. A number of factors could contribute to the explanation of the observed phenomena. Cervical dislocation may cause a widespread stimulation or disinhibition of neuronal activity in the brain with subsequent release of transmitters. Due to anoxia, re-uptake may fail, neuronal membranes start to disintegrate and the extracellular concentration of transmitters will rise. Metabolism is probably also hampered because of the failing reuptake and lack of oxygen (both requisites for dopamine deamination by MAO). The results indicate a rapid massive release of DA in the brain immediately following death. This suggests that the releasable and non-releasable pools of endogenous DA may be dramatically altered by a large post-mortem redistribution of the neurotransmitter. Furthermore, DA (auto)receptors and coupled mechanisms (like tyrosine hydroxylase activity) may be changed as a consequence of a massive postmortem receptor stimulation.
[Arnold G. Vulto was in receipt of a European Training Programme in Brain and Behaviour Research short-term fellowship]

353.8 PROLACTIN-INDUCED ACTIVATION OF TUBEROINFUNDIBULAR DOPAMINE NEURONS IS MEDIATED BY THE ANTERIOR HYPOTHALAMUS. Anne C. Gredler and Keith T. Demarest. Departments of Pharmacology/Toxicology and Physiology, Michigan State University, East Lansing, MI 48824

The neurosecretory activity of tuberoinfundibular dopamine (TIDA) neurons is regulated by the feedback of circulating levels of prolactin (Neuroendocrinology 38:467, 1983). Previous studies have demonstrated sexual differences in the activity of TIDA neurons which are due, at least in part, to a difference in the responsiveness of TIDA neurons to the action of prolactin (Neuroendocrinology 33: 230, 1981). The present study was undertaken to determine whether the action of prolactin to stimulate TIDA neuronal activity is mediated by an intra- vs extra-hypothalamic origin and if anterior hypothalamic influences differ in male vs female rats. In these studies the effect of complete and partial hypothalamic deafferentation on the neurosecretory activity of TIDA neurons and their responsiveness to prolactin was examined. Hypothalamic deafferentations consisted of a complete medial basal hypothalamic island (MBH lesion) or an extended anterior retrochiasmatic cut (RC lesion) (Endocrinology 80: 608, 1967). The synthesis and turnover of DA in the median eminence was used as an index of TIDA neurosecretory activity and was estimated by the rate of DOPA accumulation following NSD 1015 (100 mg/kg, i.p.) or by the alpha-methyltyrosine (AMPT; 250 mg/kg free base i.p.) induced decline of DA after 0, 30 and 60 min. Both the MBH and RC lesions decreased the rate of DOPA accumulation and DA turnover in the median eminence of female, but not male rats. These results suggest that in the female rat TIDA neuronal activity is tonically stimulated via an anterior hypothalamic afferent influence, while this influence is either absent or repressed in the male. Since previous studies have demonstrated that TIDA neurons in female rats are tonically stimulated by circulating prolactin, these results may suggest that the action of prolactin to stimulate TIDA neurons is mediated via the anterior hypothalamus. In support of this hypothesis is the demonstration that the increase in DOPA accumulation in the median eminence, induced by either haloperidol (2.5 mg/kg, s.c.; 16 h prior to sacrifice) or ovine prolactin (10 mg/kg, i.p. x 3 d) administration, was blocked by a RC lesion in both female and male rats. These results suggest that the greater activity of TIDA neurons in the female rat is the consequence of a tonic stimulatory influence from the anterior hypothalamus. In addition, the action of prolactin to stimulate TIDA neurons in both male and female rats appears to be mediated via an anterior hypothalamic pathway. (Supported by NIH grant AG02644).

353.9 HEMORRHAGE INCREASES THE NEUROSECRETORY ACTIVITY OF TUBEROHYPOPHYSEAL DOPAMINE NEURONS. Keith T. Demarest. Depts. of Pharmacology/Toxicology and Physiology, Michigan State Univ., E. Lansing, MI 48824

Previous studies have demonstrated that several days of dehydration (Neuroendocrinology 31: 112, 1980) or acute injections of hypertonic saline (Neuroendocrinology 35: 469,1982) increase the rate of dopamine (DA) synthesis in the neurointermediate lobe (NIL) of the pituitary. Thus the activity of tuberohypophyseal (TH) DA neurons appear to be regulated, at least in part, by sodium or osmoreceptors. The present studies were undertaken to determine if hemorrhage, which alters blood volume and pressure, influences the synthesis and turnover of DA in the NIL. Male rats were hemorrhaged under ether anesthesia via a retrograde orbital sinus puncture of 1-2 min duration. The synthesis of DA in the NIL, median eminence and striatum was estimated by measuring the rate of dihydroxyphenylalanine (DOPA) accumulation 30 minutes after the administration of the decarboxylase inhibitor, NSD 1015 (100 mg/kg, i.p.). The turnover of DA and norepinephrine (NE) was determined by the rate of the alpha-methyltyrosine (AMPT; 250 mg/kg free base i.p.) induced decline of amine concentrations after 0, 30 and 60 minutes. The rate of DOPA accumulation in the NIL increased within 30 minutes following a hemorrhage of 1.5 ml/kg (0.75 $\pm$ 0.08 to 1.21 $\pm$ 0.08 ng/mg protein), but returned to control values by 4 h after the onset of the hemorrhage. This treatment did not alter DA or NE concentrations in the NIL or other regions examined. The rate of the AMPT-induced decline of DA increased 2-3 fold in the NIL (0.37 $\pm$ 0.02 vs 1.11 $\pm$ 0.04 ng/mg protein/h) immediately following a hemorrhage of 1.5 ml/kg, while that of NE was unchanged (0.14 $\pm$ 0.02 vs 0.12 $\pm$ 0.02 ng/mg protein/h). No change in the rate of DOPA accumulation or DA and NE turnover was observed in the median eminence or striatum at anytime after hemorrhage confirming that this treatment was selective for the NIL and did not alter the absorption or distribution of either NSD 1015 or AMPT. In addition, the ether anesthesia and orbital sinus puncture alone without hemorrhage were without effect. The hemorrhage-induced increase in TH DA neuronal activity was volume dependent; increasing the volume bled (0.5-2.0 ml/kg) resulted in a graded increase in DOPA accumulation in the NIL. These results demonstrate that hemorrhage induces an acute selective increase in the neurosecretory activity of TH DA neurons. This study also suggests that, in addition to osmoreceptor mediated mechanisms, the TH DA system may be regulated by a baroreceptor mechanism(s). (Supported by NIH grant AG02644).

353.10 LONG-TERM ESTRADIOL TREATMENT INDUCES IRREVERSIBLE DAMAGE TO TUBEROINFUNDIBULAR DOPAMINERGIC NEURONS IN FISHER 344 RATS BUT NOT IN LONG-EVANS RATS. Gail D. Riegle*, Kenneth E. Moore, Kathryn L. Lovell, and Keith T. Demarest (SPON: G.M. Lew). Depts of Pharmacology/Toxicology, Physiology and Anatomy, Michigan State Univ., E. Lansing, MI 48824

Previous studies have demonstrated that long-term estradiol treatment of female ovariectomized Long-Evans rats induces reversible alterations in tuberoinfundibular dopaminergic (TIDA) neurons which are characterized by decreased DA content and synthesis in the median eminence and by a decrease in the responsiveness of these neurons to prolactin (Neuroendocrinology 39: 193, 1984). Since other investigators have suggested that estradiol treatment induces irreversible damage to TIDA neurons in Fisher 344 rats, the present studies were undertaken to compare the effects of the long-term estradiol treatment of intact female rats of the Long-Evans versus Fisher 344 strains. Intact female rats of both strains were implanted with silastic capsule containing estradiol benzoate (5 mm exposed length of 0.062 in ID, 0.175 in OD silastic tubing). Estradiol treatment increased serum prolactin concentrations in both strains. This increase was of a 5-10 fold greater magnitude in the Fisher 344 rats. The increase in prolactin was associated with a dramatic increase in anterior pituitary size; the increase was 3-4 fold larger in the Fisher 344 rats. Estradiol treatment decreased the concentrations of DA and the rate of DA synthesis in the median eminence by 36 days in both strains. No change in norepinephrine (NE) concentrations were observed in the median eminence of Long-Evans rats, but a dramatic decrease was observed in the Fisher 344 rats. Similarly, DA-NE content and DA synthesis in the neurointermediate lobe of the pituitary (NIL) were not changed in the Long-Evans rats, but were decreased in the Fisher 344 rats. These changes in NE content in the median eminence and DA-NE content in the NIL are apparently non-selective and are most likely due to the severe compression associated with the estradiol-induced hypertrophy of the anterior pituitary as demonstrated by histological analysis. The DA concentration in the median eminence of Long-Evans rats returned to control values by 18-36 days following removal of the estradiol-containing capsule. DA and NE content in the median eminence and NIL of the Fisher 344 rats was decreased following termination of estradiol treatment for as long as 36 days. These results suggest that the irreversible damage associated with long-term estradiol treatment in the Fisher 344 rats is most likely due to the hypertrophy of the anterior pituitary gland and not as direct "neurotoxic" action of estradiol or prolactin on TIDA neurons. (Supported by NIH grant AG02644 and NS 09174).

353.11 DIFFERENTIAL RESPONSE OF TUBEROINFUNDIBULAR DOPAMINE NEURONS TO CASTRATION IN MALE VERSUS FEMALE RATS. Mary Lynn Bajt and Keith T. Demarest. Depts. of Pharmacology/Toxicology and Physiology, Michigan State Univ., E. Lansing, MI 48824

Previous studies have demonstrated a greater rate of DA synthesis and turnover in the median eminence of female versus male rats which is due, at least in part, to a greater responsiveness of tuberoinfundibular dopamine (TIDA) neurons in the female rat to the stimulatory actions of prolactin (Neuroendocrinology 32: 108, 1981; 33: 230, 1981). The present studies were undertaken to determine the effect of endogenous gonadal steroids on the activity of TIDA neurons. Adult male and female rats of the Long-Evans strain were castrated 1 week prior to sacrifice. Intact female rats were sacrificed on the second day of diestrus. The synthesis of DA was estimated by measuring the rate of dihydroxyphenylalanine (DOPA) accumulation 30 minutes after the administration of NSD 1015. The turnover of DA and norepinephrine (NE) was determined by the rate of the alpha-methyltyrosine-induced decline of amine concentrations after 0, 30 and 60 minutes. DA synthesis was approximately 3 times greater in the median eminence of intact female vs male (16.8 ± 2.1 vs 4.8 ± 0.9). Following castration, the rate of DA synthesis in the median eminence of female rats was decreased but in male rats it was increased; resulting in similar rates of DA synthesis in the castrate male and female groups. Following castration, the rate of DA turnover in the median eminence of female rats was unchanged while the rate of NE turnover increased 2 fold. In male rats, castration caused a 2 fold increase in both DA and NE turnover in the median eminence. These results demonstrate a differential effect of castration on DOPA accumulation in the median eminence of male vs female rats. In the male, the castration induced increase in DOPA accumulation appears to be the result of an increase in both DA and NE turnover. However, the decrease in DOPA accumulation in the female is not consistent with the lack of change in DA turnover and the apparent increase in NE turnover. The changes in DA turnover in the female are somewhat confounded by an increase in DA steady-state concentrations associated with castration which is not observed in the male. The observed male-female differences in TIDA neuronal activity cannot be explained on the basis of differences in circulating prolactin concentrations since serum prolactin concentrations, determined in a separate group of rats, were not dramatically different between intact versus castrate female. These results suggest that the male-female difference in TIDA neuronal activity may be mediated by the presence of endogenous gonadal steroids. (Supported by NIH grant AG02644).

353.12 EFFECTS OF 3-PPP ENANTIOMERS, SKF-38393, AND 8-OH-DPAT ON SYNTHESIS IN MESOCORTICAL DOPAMINE NEURONS, D. Clark*, and M.P. Galloway, (SPON: M. Stanley), Laboratory of Neurophysiology, Center for Cell Biology, Sinai Hospital, Detroit, MI 48235, and Neurochemical Pharmacology Research Unit, Lafayette Clinic, Dept. of Psychiatry, Wayne State University, Detroit, MI 48207

Biochemical and electrophysiological evidence suggests that mesencephalic dopamine (DA) neurons projecting to the medial prefrontal cortex (MPF), unlike those innervating and striatum (STR) and limbic structures, lack functional terminal autoreceptors regulating DA synthesis, and somatodendritic DA autoreceptors controlling impulse flow. Although low doses of apomorphine can inhibit DOPA accumulation in MPF cortex, it has been proposed that this effect on synthesis is mediated indirectly by activation of DA release modulating autoreceptors with a subsequent increase in end-product inhibition of tyrosine-3-monooxygenase. In light of the possible functional implications of regulatory differences between DA systems, we have compared the regional effects of other monoamine agonists on DA synthesis.

The D2 agonist (+)-3-PPP (0.5-5.0 mg/kg, sc) reduced DA synthesis rate (measured in vivo as the accumulation of DOPA following decarboxylase inhibition by NSD 1015) in rat STR, nucleus accumbens (NAC) and MPF. In contrast the D1 agonist SKF 38393 (1-3 mg/kg) was ineffective in these areas whereas the 5HT agonists 8-OHDPAT (0.1 mg/kg) and TFMPP (1 mg/kg) slightly elevated (20-40%) DOPA accumulation. (-)-3-PPP, which acts as a partial agonist at normosensitive DA autoreceptors but as an antagonist at normosensitive postsynaptic DA receptors, exerted variable actions on DA synthesis. A low dose of (-)-3-PPP (0.5 mg/kg)reduced NAC DOPA but no effect was observed after 5.0 mg/kg. In the STR, DOPA was not influenced by the low dose of (-)-3-PPP and actually increased after the higher dose. In contrast, DOPA accumulation in the MPF was uniformly inhibited by both doses of (-)-3-PPP. In animals pretreated with reserpine, 3-PPP enantiomers strongly reduced STR and NAC DOPA levels but were without effect in the MPF cortex.

The fact that reserpine abolishes the inhibitory action of the 3-PPP enantiomers (and apomorphine) on MPF DA synthesis supports the hypothesis that DA agonists inhibit MPF synthesis indirectly via stimulation of D2 autoreceptors regulating DA release. The different effects of (-)-3-PPP in the MPF of normal animals, relative to those in the STR and NAC, may suggest that DA sensitive long-loop feedback pathways exert only a weak influence on impulse flow of mesoprefrontal DA neurons. Supported by Dept. of Mental Health, State of Michigan.

353.13 DOSE-RESPONSE AND TIME COURSE CHARACTERISTICS OF THE DOPAMINERGIC RESPONSE TO ACUTE COCAINE. S. M. Lasley, M. W. Emmett-Oglesby and J. D. Lane. Dept. of Pharmacology, Texas College of Osteopathic Medicine, Ft. Worth, TX 76107.

The primary mechanism of cocaine (COC) action is thought to involve an interaction with the monoamine synaptic reuptake site, particularly at dopaminergic nerve terminals. However, much evidence in support of this proposal is based on in vitro studies in which it is difficult to distinguish the releasing and reuptake blocking properties of an agent. Furthermore, a systematic in vivo analysis of the dose-response and time course characteristics of dopamine (DA) metabolism after acute administration of COC is not available, and would further elucidate the drug's effects. Such data is necessary for further studies investigating the changes resulting from chronic COC administration.

Separate groups of 100-day old male Long-Evans hooded rats were given i.p. injections of COC in doses ranging from 1.25-40 mg/kg and were sacrificed 20 min later. Additional groups of animals were injected i.p. with 10 mg/kg COC and sacrificed at periods 10-60 min post-injection. Brains were dissected at -20°C and medial prefrontal cortex, nucleus accumbens, caudate nucleus, hypothalamus, substantia nigra, and ventral tegmental area removed for quantitation of DA, dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) by liquid chromatography with electrochemical detection. COC levels in plasma were determined in the same rats by gas chromatography with thermionic detection.

At higher doses or intermediate time intervals DOPAC content was decreased and HVA content was increased in a manner consistent with COC's actions as a reuptake blocker. However, at lower doses or shorter time intervals DOPAC concentrations were increased while HVA concentrations were essentially unchanged, suggesting that the drug also possesses releasing properties. Increases in DA content in nucleus accumbens and caudate nucleus did not exceed 15% and were substantially less than those that have been observed with d-amphetamine, another psychomotor stimulant. Plasma levels of COC increased linearly from 0.07 µg/ml at the lowest dose to 2.73 µg/ml at the highest dose. Subsequent work will compare these changes to those occurring after various protocols of chronic COC administration.

353.14 EFFECTS OF ACUTE $\Delta^9$-TETRAHYDROCANNABINOL (THC) EXPOSURE ON HYPOTHALAMIC NEUROTRANSMITTER SYSTEMS CONTROLLING PROLACTIN AND GONADOTROPIN RELEASE. S. Dalterio and R. Steger. Departments of Pharmacology and OB-GYN, University of Texas Health Science Center, San Antonio, Texas 78284.

Administration of THC, the major psychoactive component of marihuana, markedly inhibits circulating androgen, gonadotropin and prolactin levels in male and female mice. In previous studies we have shown that in addition to its effects in decreasing levels of these hormones, treatment with the non-psychoactive cannabinol (CBN), also reduced the concentration of norepinephrine (NE) in the median eminence (Life Sci. 36,1299, 1985). The present experiments were conducted to determine the acute effects of a 50 mg/kg oral dose of THC on NE, dopamine (DA), serotonin (5-HT),and 5-hydroxyindole acetic acid (5-HIAA) content in specific hypothalamic regions of mouse brain. Hypothalamic amines were assayed using HPLC and electrochemical detection. In addition, the effects of THC on NE and DA turnover was estimated by measuring the depletion of these amines after the inhibition of tyrosine hydroxylase with α-methyl-p-tyrosine (α-MPT). THC significantly increased median eminence (ME) DA content and turnover (0.154 ± 0.011 vs 1.806 ± 0.258 ng DA/ ME/hr) within 60 of THC treatment in intact male mice. In castrated male mice, DA content was elevated 15 min post-treatment with THC and remained elevated for at least 90 mins. Dopamine turnover in the medial basal hypothalamus (MBH) was decreased after THC treatment in intact mice, while levels in the medial preoptic area (MPOA) were unaffected. The NE content in ME, MBH or MPOA in intact or castrated mice was not significantly affected by THC treatment, but NE turnover tended to be decreased in the ME region of intact THC-treated mice. Serotonin content was not affected by THC administration in any of these studies, but this does not rule out possible THC-induced changes in 5-HT metabolism. In the final experiment, basal or NE-stimulated (60µM) LHRH release from ME fragments incubated in vitro were not affected by oral administration of THC at 60 min before sacrifice and incubation, or by the addition of THC (250 ng/ml) to the incubation media. This data, together with that obtained after repeated CBN exposure, suggests that cannabinoid-induced inhibition of prolactin secretion may be mediated, in part, by its ability to inhibit the tuberoinfundibular dopaminergic system regulating pituitary prolactin release. The effect of THC on gonadotropin secretion may also be due, at least in part, to inhibition of hypothalamic NE metabolism, but does not appear to be due to a direct effect of THC on LHRH neurons. It is also possible that THC may affect gonadotropin secretion through changes in DA metabolism, but further studies are required to evaluate this hypothesis.

353.15 ENHANCEMENT OF CATECHOLAMINE SYNTHESIS IN Na-DEPRIVED MEDIUM IN CULTURED BOVINE ADRENAL MEDULLA CELLS.
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In adrenal medulla cells, carbachol stimulates the secretion and synthesis of catecholamines which are both dependent on extracellular Ca. Recently, we reported that carbachol caused the influx of Na to the cultured bovine adrenal medulla cells via acetylcholine receptor mediated Na-channels and that influx of Na to the cells is a requisite for influx of Ca and the secretion of catecholamines (Neuroscience Lett., 47:78, 1984, Neuroscience, in press).

In the present study, we examined the effect of Na-deprivation from the incubation medium on the synthesis of catecholamines in order to investigate the role of extracellular Na in the regulation of catecholamine synthesis. Cultured adrenal medulla cells were incubated with L-[U-$^{14}$C] tyrosine for 30 min at 37°C and formed $^{14}$C-catecholamines were separated by ion exchange column chromatography using Duolite C-25. Intracellular pH was measured by the uptake of$^{14}$C-5,5-dimethyloxazolidine -2,4,-dione.

In Na-deprived sucrose-enriched medium, synthesis of $^{14}$C-catecholamines was much enhanced, while uptake of $^{14}$C-tyrosine to the cells was reduced. Enhancement of catecholamine synthesis by Na-deprivation was observed even if Ca was omitted from the medium. Dibutyryl cyclic AMP (2mM) increased the synthesis of $^{14}$C-catecholamines irrespective of the existence of Na in the medium. In control cells, the intracellular pH was 7.16 and it was shifted to 6.75 in Na-deprived medium, a condition which favors the activity of tyrosine hydroxylase, since the optimal pH for this enzyme is known to be 5.8 - 6.3.

These results suggest that Na-deprivation from the incubation medium enhances the synthesis of $^{14}$C-catechoamines from $^{14}$C-tyrosine probably through the decreas in intracellular pH in cultured bovine adrenal medulla cells.

353.16 NICOTINIC ACETYLCHOLINE RECEPTOR-ASSOCIATED Na-CHANNELS AND Na,K-ADENOSINE TRIPHOSPHATASE IN CULTURED BOVINE ADRENAL MEDULLA CELLS.
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In adrenal medulla cells, sodium ion (Na) has been considered not to be important in the secretory process of catecholamines mediated by acetylcholine receptor. Recently, we reported that in cultured bovine adrenal medulla cells, $^{22}$Na influx via the nicotinic receptor-associated Na-channels is intimately involved in $^{45}$Ca influx, a requisite for triggerring the secretion of catecholamines (Neuroscience Lett, 47:75-80, 1984, Neuroscience, in press).

In this paper, we examined whether inhibition of Na pump by ouabain or extracellular K deprivation modulates carbachol-induced cell responses. We also measured $^{86}$Rb uptake by the cells as index of Na, K-ATPase activity. (1) Carbachol caused rapid and transient influx of $^{45}$Ca and secretion of catecholamines which were not inhibited by tetrodotoxin, but were significantly reduced in Na free medium. (2) Carbachol evoked rapid and transient influx of $^{22}$Na which was inhibited by hexamethonium and d-tubocurarine, but not by tetrodotoxin. Concentration-response curve of carbachol for $^{22}$Na influx was almost identical to that for $^{45}$Ca influx. (3) Ouabain or K removal remarkably potentiated carbachol-induced $^{22}$Na influx, $^{45}$Ca influx and secretion of catecholamines. (4) Carbachol caused an increase of $^{86}$Rb uptake to the cells. In Na free medium, carbachol did not increase the uptake of$^{86}$Rb. (5) Ouabain inhibited carbachol-induced uptake of $^{86}$Rb in a concentration-dependent manner as it caused cellular accumulation of $^{22}$Na.

These results suggest that nicotinic acetylcholine receptor-associated Na-channels and Na, K-ATPase, both modulate influx of Ca and secretion of catecholamines through a cooperative regulation of intracellular Na concentration.

353.17 NOREPINEPHRINE TURNOVER IN CEREBELLUM AND OTHER BRAIN·REGIONS OF pcd MUTANT MICE. Kenneth W. Perry*, Ray W. Fuller and Bernardino Ghetti. Lilly Research Laboratories, Eli Lilly and Company and Indiana University Medical Center, Indianapolis, IN 46285 & 46223.

In the Purkinje cell degeneration (pcd) mutant mouse, Purkinje cells in the cerebellum begin degenerating at about 17 days of age, and 99% are lost by 45 days. The Purkinje cells are target cells for brain stem norepinephrine (NE) neurons. The degeneration of the Purkinje cells leads to a decrease in cerebellar weight and an increase in NE concentration with no change in the total content of NE per cerebellum (Ghetti et al, Brain Res. Bull. 7, 711-714, 1981). We have now evaluated NE turnover in cerebellum, hypothalamus and brain stem of pcd mice by measuring MHPG (3-methoxy-4-hydroxy-phenylethyleneglycol) and the ratio of MHPG/NE and by measuring 3,4-dihydroxyphenylalanine (DOPA) accumulation after decarboxylase inhibition.

Tissues from 5-6 mice per group were homogenized in 0.1 M trichloroacetic acid, and assays were performed on the supernatant fraction. The catechols (DOPA and NE) were assayed by liquid chromatography with electrochemical detection (LCEC) after adsorption onto and elution from alumina. DOPA was measured 30 min after injection of NSD 1015 (m-hydroxybenzylhydrazine), 100 mg/kg i.p. MHPG was assayed by LCEC using Bond-Elute CH columns for preliminary isolation, then injecting the eluate onto the C18 column with 0.1 M Na2PO4, 0.05 M citric acid as a mobile phase.

The content of MHPG in cerebellum and the ratio MHPG/NE were decreased in 3, 6 and 9 month old pcd mice compared to controls. The content of NE (amount per cerebellum) was unchanged, but the concentration of NE was increased due to the reduction in cerebellar weight in pcd mice. DOPA accumulation was decreased in cerebellum to 69%, 53% and 37% of control values, respectively, at 3, 6 and 9 months in pcd mice, but was not changed in brain stem and was only slightly decreased in hypothalamus. No decrease in MHPG (content or concentration) or in the ratio MHPG/NE was found in brain stem. In hypothalamus, NE concentration and content were slightly increased in pcd mice at all ages, and the ratio MHPG/NE was decreased in 6 and 9 month old pcd mice.

Although NE axons in the cerebellum are maintained in pcd mice after Purkinje cell degeneration, NE turnover was decreased. NE turnover was decreased slightly in hypothalamus but not in brain stem, though in both regions NE concentration was increased slightly. Apparently the NE projections to the cerebellum are intact, but the NE neurons do not synthesize and release their neurotransmitter at normal rates when their target Purkinje cells have degenerated. Since NE may act as a modulator in conjunction with other neurotransmitters, these data might suggest that some regulatory mechanisms of NE neurons operate to decrease function of the cerebellar NE axons when target cells are lost.

353.18 PHENYLETHANOLAMINE N-METHYLTRANSFERASE CONTAINING CELLS IN RAT RETINA: IMMUNOHISTOCHEMISTRY, IMMUNOCHEMISTRY AND MOLECULAR BIOLOGY. D.H. Park, G. Teitelman, M. Evinger, J. Woo*, D. Ruggiero, V. Albert, E. Baetge, V. Pickel, D. Reis and T.H. Joh. Depart. of Neurology, Cornell Univ. Med. Coll., New York, NY 10021.

Biochemical and indirect immunofluorescence histochemistry indicated the presence of epinephrine synthesizing enzyme, phenylethanolamine N-methyltransferase (PNMT) in the inner nuclear layer (INL), inner plexiform layer (IPL) and ganglion cell layer (GCL) of rat retina (Hadjiconstantinou et al., Neurosci., 1984). It has been reported that hypophysectomy resulted in decrease of adrenal PNMT but no change of retinal PNMT. In order to further characterize these cells, we investigated: (1) the anatomical details of PNMT neurons using PAP-immunohistochemical techniques and antibody double label methods; (2) whether other catecholamine biosynthetic enzymes, specifically tyrosine hydroxylase (TH), DOPA-decarboxylase (DDC), and dopamine B-hydroxylase (DBH), are present in the same cells; (3) whether retinal PNMT possesses immunochemical and biochemical properties similar to adrenal PNMT; and finally (4) whether PNMT mRNA can be identified in the retina, thereby confirming PNMT biosynthesis in retina. Immunohistochemistry using bovine adrenal PNMT antibody showed that cell bodies containing PNMT as the only CA-synthesizing enzyme were mainly localized in the INL of retina. A small population of exclusively PNMT-positive cell bodies were also observed in the GCL. Both PNMT cell bodies in the INL and GCL sent their dense processes to the IPL. Lightly stained PNMT-containing varicosities from cells of the INL were distributed in the outermost stratum of the IPL. Antibody double labeling method was used to determine whether other catecholamine enzymes, namely TH, DDC and DBH, coexist with PNMT in these cells. The fact that antibodies to other catecholamine enzymes failed to stain these cells indicated that these cells are PNMT specific cells. The exception was a small percentage of PNMT-staining cell bodies in the INL which were stained for TH and DDC, but not DBH. These cell bodies send their fibers to the outermost striatum of the IPL. PNMT ac tivity in rat retina was 10 pmole/mg prot./15 min at 37°C (Sprague Dawley, male rats). Although hypophysectomy did not alter PNMT activity in retina unlike adrenal enzyme, polyclonal antibodies raised in rabbits against bovine adrenal PNMT cross reacted with rat retina PNMT in similar fashion as with bovine adrenal PNMT in immunochemical titration experiment. The presence of PNMT mRNA in the retina as well as adrenals was identified by dot blot analysis using $^{32}$P-labeled PNMT cDNA probe. Thus, we conclude that the majority of the PNMT containing cells in the rat retina are PNMT specific cells, which do not contain other catecholamine enzymes. However, only the small portion of PNMT-containing cells in the INL contain the other CA-synthesizing enzymes TH and DDC but not DBH. Biochemical and PNMT cDNA dot blot analyses confirm that PNMT protein synthesis occurs in retina. (Supported by NIH grants, NS19002 and HL18974, and NIMH grant, MH24285.)

353.19 TIME COURSE OF THE DEPLETION OF BRAIN AND HEART NOREPINEPHRINE AFTER INTRAVENOUS ADMINISTRATION OF DSP4. M.C. Bennett, B.J. Vasquez, P. McNeeley*, L. Cahill* and J.L. McGaugh. Center for the Neurobiology of Learning and Memory and the Department of Psychobiology, University of California, Irvine, CA 92717 and V.A. Med. Center and Loma Linda University, Loma Linda, CA 92357.

Male CFW mice, 60 days of age, were housed (6 per cage) in an environmentally-controlled laboratory (LD 12:12, light on at 7 a.m.) with food and tap water available ad libitum. One week after arrival, the mice were injected i.v. (tail vein) with 25 mg/kg of the noradrenergic neurotoxin DSP4 (Hoechst). The drug was dissolved in cold physiological saline (2.5 mg/ml) in aliquots sufficient to inject one group of six mice and it was kept on ice during the entire procedure. Groups of six mice each were sacrificed 1, 2, 4, 12, 24 and 48 hours, 7, 14 and 28 days after injection. A control group which received a volume of 10 ml/kg saline was sacrificed one hour after injection. All animals were sacrificed between 10 a.m. and 12 noon to minimize the circadian variability in monoamine levels.

Monoamine levels were assayed in brain (minus cerebellum) and heart tissue by HPLC-EC. The tissues were briefly sonicated at minimum power in cold 0.1 M perchloric acid containing sodium metabisulfite. After centrifugation the supernatants were analyzed on a reverse phase ultrasphere C-18 column (Altex) and an LC-4A amperametric detector (BAS, Inc.) at an applied voltage of +0.7V. The mobile phase used was a citrate-phosphate buffer (PH4) with 10% methanol.

A significant reduction of norepinephrine (NE) was found in brain and heart tissue within 4 hr of DSP4 administration. Brain NE levels remained significantly depleted at the last time point measured, 28 days, while the peripheral tissue recovered to control levels within 14 days posttreatment.

The depletion from the 25 mg/kg i.v. dose of DSP4 was more robust and less variable than that found previously after 50 mg/kg i.p. administration. Further, large and significant depletion was found within hours of drug treatment. These results suggest that behavioral measurements may be done within hours, not days, after drug treatment, thus making them more comparable to those obtained after treatment with drugs that produce acute depletion of NE. Moreover, such findings may be less likely to be confounded by denervation supersensitivity.

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353.20 EFFECTS OF DIFFERENT STRESS PARADIGMS ON CENTRAL DOPAMINE AND NOREPINEPHRINE METABOLISM. J.-X. Yang*, A. M. Knorr*, Kenan Onel*, S.-Y. Tam, A. Y. Deutch, L. Lubich* and R. H. Roth (SPON: J. A. Holley), Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut 06510

The mesocortical dopamine (DA) system has been implicated in emotional responses to stress. Stress paradigms such as footshock and conditioned fear increase DA metabolism in the prefrontal cortex (PFC), while the mesolimbic and nigrostriatal DA systems are largely unaffected. We have examined the effects of two other stressors on central DA metabolism and compared the results to those observed in response to footshock or conditioned fear paradigms. Changes in central norepinephrine (NE) metabolism under the various stress paradigms were assessed by measuring levels of the NE metabolites, DHPG and MHPG, by GC/MS.

Rats subjected to a 5-minute swim stress exhibited a significant increase in levels of the DA metabolite DOPAC in the PFC; other mesocortical, mesolimbic and nigrostriatal areas were unaffected. In the restraint stress paradigm a significant and selective increase in PFC DOPAC levels was observed. Thus, both the swim stress and restraint paradigms effected selective increases in PFC DA metabolism. This selectivity is similar to that observed in both footshock and conditioned fear paradigms: DOPAC levels are increased in the PFC and ventral tegmental area (source of the mesocortical DA innervation), but not altered in mesolimbic or nigrostriatal areas. Furthermore, the selective increase in PFC DOPAC levels is prevented by pretreatment with diazepam.

Both the swim stress and restraint procedures resulted in specific and selective changes in central NE metabolism. Swim stress effected a selective increase in DHPG, but not MHPG, levels in the hypothalamus. In contrast, both NE metabolites in the hypothalamus were elevated following restraint. Neither DHPG nor MHPG levels were altered in dorsal-bundle innervated regions, such as the PFC. The swim stress-elicited increase in DHPG levels was prevented by subacute treatment with tricyclic antidepressants.

These studies demonstrate that the mesoprefrontal DA system is uniformly activated under all four stress paradigms examined. The central noradrenergic systems, in contrast, do not respond to mild footshock stress, but are regionally activated by swim stress or restraint. Observed alterations in NE metabolism are confined to the ventral NE hypothalamic projection field. These data therefore suggest that the prefrontal cortical dopaminergic system may be critically involved in stress activation, even under such mild conditions that minimal hypothalamic-hypophyseal noradrenergic activation is observed. These data further indicate that the prefrontal cortical activation in response to stress exposure is confined to the DA system and does not involve concomitant changes in PFC NE systems. (Supported by MH 14092 and the State of Conn.)

353.21 NEUROTRANSMITTER TURNOVER RATE CHANGES IN BRAIN REGIONS OF RATS EXPOSED TO PUNISHMENT. T. Miyauchi*, S.I. Dworkin, C. Co* and J.E. Smith (SPON: G.F. Guerin). Psychiatry Research Unit, Departments of Psychiatry and Pharmacology, Louisiana State University Medical Center, Shreveport, LA 71130.

The neurobiological effects of punishment are not well understood. Several of the difficulties in such investigations involve controlling for response rate, reinforcement density and the direct effects of the punisher. This experiment determined neurotransmitter turnover rates in brain regions of rats exposed to punishment and controlled for the above non-specific factors.

Eight triads of male littermate rats were used. One littermate was trained on a conjoint random-ratio food, random-ratio shock schedule while the second rat's random interval schedule of food presentation was yoked to the subject on the ratio contingency. A third rat received food and shock response independently when ever they were presented to the subject on the ratio contingencies. Similar rates of responding and reinforcement densities were obtained from the rats on the punished-ratio and unpunished-interval contingencies while the third littermate was a control for the nonspecific effects of food and electric shock. The subjects were trained on the procedures and then implanted with chronic jugular catheters. After recovery of baseline responding the subjects were injected with 1 mCi [$^{3}$H]-tyrosine, 0.5 mCi [$^{3}$H]-tryptophan and 0.2 mCi [$^{14}$C]-glucose through jugular catheter immediately before the behavioral session. After either 60 or 90 min exposure to the appropriate behavioral condition, they were sacrificed by total freezing in liquid nitrogen. Brains were removed and dissected into 22 brain regions and the turnover rates of dopamine, epinephrine, norepinephrine, serotonin, aspartate, glutamate and gamma-amiobutyric acid determined by high-pressure liquid chromatography and liquid scintillation spectrophotometry. Significant changes in turnover rates of specific neurotransmitters in discrete brain regions were correlated with the different behavioral conditions. Discrete neuronal pathways and circuits may mediate punished responding. (Supported in part by USPHS Grant DA-01999).

354.1 DIFFERENTIATION OF STRIATAL NEURONS IN PRIMARY CULTURE. S. Weiss*, M. Sebben*, J.P. Pin*, F. Sladeczek*, P. Greengard, J. Gabrion* and J. Bockaert* (SPON : C. Chevillard) Centre CNRS-INSERM de Pharmacologie-Endocrinologie, BP 5055, 34033 Montpellier Cedex France and Laboratory of Molecular and Cellular Neuroscience, Rockefeller University, New York, NY 10021, U.S.A.

The biochemical and morphological properties of striatal neurons, generated from the foetal mouse brain and raised in primary culture, were examined. Recent advances in our laboratory now permit the maintenance of these neurons for 18-21 days in vitro (DIV), in a serum-free medium, devoid of non-neuronal cell types. Electron microscope (EM) observations revealed a synthesis and axonal migration of synaptic vesicles within 3 to 6 DIV. After 10-11 DIV synapse formation was observed, as determined by the presence of vesicles in nerve terminals and pre- and post-synaptic densities between contacting structures. These findings were complemented with the immunofluorescent detection of Synapsin I. After 3 DIV Synapsin I was present only in nerve cell perikarya. At 6 DIV immunostaining was detectable in both perikarya as well as in varicosities along extended axons. After 11-13 DIV, Synapsin I was undetectable in perikarya, yet displayed a concentrated immunofluorescence at contact points between axon terminals and post-synaptic sites on dendrites and perikarya.

Neurotransmitter-stimulated biochemical responses were studied by examination of intracellular cyclic AMP and inositol phosphate formation in intact neurons. Although clearly detectable within 3 DIV, the formation of both putative second messengers increased dramatically between 6 and 10 DIV, at which point the response reached a plateau. Endogenous GABA release from striatal neurons was examined with HPLC analysis. $K^+$ (56mM) depolarization resulted in a 2-fold increase in GABA release, 50 % of which was $Ca^{2+}$ dependent, between 3-11 DIV. Between 11-14 DIV, subsequent to synapse formation revealed by EM, release evoked by 56 mM $K^+$ increased up to 5-fold, 75% of which was $Ca^{2+}$-dependent.

The findings of this study suggest that striatal neurons in primary culture, grown in serum-free media, provide an excellent model for the study of the physiological and regulatory mechanisms involved in nerve cell development.

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354.2 DISTRIBUTION OF CALCINEURIN-IMMUNOREACTIVITY IN DEVELOPING RAT BRAIN. K.L. Valentino and L.F. Reichardt. Dept. Physiol., UCSF, San Francisco, 94143.

Calcineurin, a $Ca^{++}$-dependent/calmodulin activated protein phosphatase, has been shown to exist in high levels in bovine neostriatum (Wallace et al., Biochem. 19: 1931, 1980) and is associated with postsynaptic densities in a certain population of synapses (Wood et al., J Cell Biol. 84:66, 1980, Valentino et al., J Histochem. Cytochem., in press). In an attempt to determine the role this phosphatase might play in synapse formation, immunocytochemical studies were performed in postnatal rats, using a polyclonal antiserum to both subunits of the enzyme.

Neonatal and adult rats were perfused with 4% paraformaldehyde and 0.1% glutaraldehyde in 0.1M Na cacodylate buffer. Following postfixation, brains were placed into 30% sucrose prior to cryostat sectioning or embedded in Lowicryl for electron microscopic studies. Fresh brains were homogenized in .32M sucrose, 0.1uM pepstatin, 2mM EDTA in 25mM Tris buffer. Samples were run on 10% SDS-PAGE gels and transferred to nitrocellulose paper. Detection of antibodies on sections and blots was with peroxidase conjugated second antibodies.

In adult rats immunoperoxidase staining of frozen sections with anti-calcineurin was heaviest in the neostriatum. In developing rats, the amount of immunoperoxidase staining in the neostriatum gradually increased to adult levels during the second and third postnatal weeks.

Immunocytochemical staining of calcineurin could also be detected in adult rat olfactory bulb. The heaviest calcineurin immunoreactivity was found in the external plexiform layer, with some staining of the glomerular layer and of some mitral cell bodies. Immunoblots of homogenates of adult olfactory bulb showed the presence of calcineurin. Calcineurin immunoreactivity could also be detected in newborn rats. At this age, heaviest staining appeared in the olfactory bulb, especially the developing external plexiform layer, and in the anterior olfactory nucleus. The presence of calcineurin in newborn brain was confirmed by immunoblots of homogenates of both whole brain and olfactory bulb and cortex. Both the phosphatase subunit and $Ca^{++}$ binding subunit of calcineurin were detected on the blots. Electron microscopic studies are in progress to determine the precise localization of calcineurin in both the adult and neonatal olfactory bulb.

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354.3 NEUROFILAMENT PHOSPHORYLATION IS A LATE EVENT FOLLOWING NEUROFILAMENT EXPRESSION IN DEVELOPMENT AND REGENERATION. D. Dahl and A. Bignami, Harvard Medical School and Veterans Administration Medical Center, West Roxbury, MA 02132.

Axon specific neurofilament (NF) antibodies, i.e. antibodies not decorating NF in perikarya and dendrites, have been reported in this laboratory (Dahl et al. J. Comp. Neur. 195:659 1981). Axon specific polyclonal and monoclonal antibodies staining NF 150 kDA and/or NF 200 kDa on immunoblots were shown to react with phosphorylated epitopes according to the criteria of Sternberg and Sternberg (Proc. Natl. Acad. Sci. USA 80:6126, 1983). The staining was abolished by dilution of the antibodies in phosphate buffer and by digestion of the sections with phosphatases. Conventional monoclonal and polyclonal antibodies, i.e. antibodies decorating NF regardless of their location (axons, perikarya and dendrites) were not affected by these treatments with one exception. On cryostat sections trypsin abolished the NF immunoreactivity of both axon-specific and conventional antibodies. Phosphatase treatment did not restore the staining. Compared to conventional NF antibodies, staining with axon-specific NF antibodies was a late phenomenon in chick embryo development. The distribution of immuno-reactive material was studied in cryostat sections double labeled with NF antibodies raised in 2 different species, i.e. axon-specific mouse antibodies and rabbit conventional NF antisera. Axon-specific immunoreactivity was first observed in peripheral nerves and in the anterior columns of the spinal cord on day 10. Sensory ganglia and optic nerve fibers were negative. With conventional NF antibodies, these structures were stained on day 4 and 5, respectively. Up to day 16, bundles of thin peripheral nerve fibers, strongly decorated by conventional NF antibodies, did not stain with axon specific antibodies in double labeling experiments. Nerve bundles emerging from sensory ganglia were also negative. Axon-specific immunoreactivity was first observed on day 17 in the optic nerve and in the layer of optic nerve fibers. At this time, staining was confined to the bundle emerging from the temporal side of the retina. Another finding was the late appearance of axon-specific immunoreactivity in regenerated nerve. As a specific example, on day 10 the distal stump of crushed rat sciatic nerve was filled with bundles of regenerating axons strongly stained with conventional NF antisera. Only few of these bundles reacted with axon-specific antibodies. (In the proximal stump as in normal nerve, the distribution of immunoreactivities was the same). Whether NF phosphorylation plays a role in axonal maturation, remains a matter for future investigation. Supported by NIH grant NS 13034 and by the Veterans Administration.

354.4 DEVELOPMENT OF A NEURONAL PROTEIN DATABASE USING PC12: THE EFFECTS OF NERVE GROWTH FACTOR (NGF) AND GLUCOCORTICOIDS. M. McTigue*, S. Halegoua*, and M.C.Bohn (SPON:J.Levine) Department of Neurobiology & Behavior, State University of New York, Stony Brook, New York 11794.

The PC12 clonal cell line synthesizes catecholamines and displays other characteristics of sympathetic neurons. For example, these cells respond to NGF by extending neuronal processes and increasing the synthesis and activity of specific neural proteins. PC12 have been used extensively to study expression and regulation of neuronal phenotypes. To identify and study proteins important in the establishment and maintenance of neuronal characteristics, we are using PC12 and quantitative 2-D gel electrophoresis to create a protein database.

Protein metabolic labeling can be quantitatively analyzed using newly developed computer techniques following separation of proteins by 2-D gel electrophoresis (Garrels, J., Meth. Enzymol. 100, 411;1983). The ability to make composites of protein patterns from different gels allows the analysis of up to 3,000 proteins. Differences in the metabolism of each protein in cells grown under different conditions can be quantified by comparing gel composites. Consequently, protein databases for identified cells can be established.

Our initial experiments to set up a neuronal protein database were carried out in PC12 cells grown under different conditions in the presence of 35S-methionine. The effects of NGF and glucocorticoids on protein metabolism were determined. To define the optimal growth conditons for labeling and treatment of the cells by these agents, the PC12 growth state was defined with respect to passage number and days within a passage for cells grown with serum or in defined medium. Alterations in the metabolism and post-translational modification of specific proteins resulting from different growth conditions and exposure to NGF or glucocorticoids will be presented.

These data provide a framework for identifying proteins specific to the nervous system. They also establish a basis for future investigation of other factors important in nerve cell differentiation and regulation.

Supported by Protein Databases, Inc., the Center for Biotechnology, S.U.N.Y. at Stony Brook, and the New York State Science and Technology Foundation.

354.5 UNIQUE NUCLEUS-ASSOCIATED PROTEINS IN DIFFERENT CELL TYPES IN THE DEVELOPING AND ADULT BRAIN REVEALED WITH MONOCLONAL ANTIBODIES. E. Pawlak-Byczkowska and P. Levitt. (SPON: A. Grigonis). Dept. Anatomy, Medical College of Pennsylvania, Philadelphia, Pa. 19129.

Non-histone chromosomal proteins have been suggested to participate in the regulation of cell differentiation in several organ systems, including the central nervous system (CNS). In order to determine the extent of unique nucleus-associated proteins in the developing brain, monoclonal antibodies were generated against sonicated, histone-depleted nuclei from fetal and adult rat brain. Both in vivo and in vitro immunization protocols were used for production of hybridomas. The supernatants from the clones were screened immunocytochemically on paraformaldehyde-fixed frozen sections from fetal rat brain to determine the cellular distribution of the antigens. The antibodies were classified into two principal groups; one set recognized antigenic determinants specific to cell nuclei and the second crossreacted with antigens present in both nuclei and cell processes. Of the nucleus-specific antibodies, one stained in a punctate pattern in mitotic and postmitotic cells of late gestation fetal cortical and subcortical regions. This same antibody was completely unreactive in all areas of the adult brain. A second nucleus-specific antibody stained a small number of cells scattered in the ventricular and subventricular zones of the late fetus. We suggest that this antibody marks a glial precursor cell because the large number of immunoreactive cells in the adult reside almost entirely in white matter. Monoclonal antibodies comprising the second principal group also displayed characteristic changes during development. Two antibodies revealed different nuclear staining patterns in similar and widespread populations of fetal mitotic and postmitotic cells. Only one continued to stain adult brain cell nuclei. These same antibodies were immunoreactive with radial glial fibers and both continued to stain astrocytes in the adult, consistent with the suggestion that nuclear and cytoscaffold proteins can share antigenic determinants. Other antibodies demonstrated a similar relationship among neuronal fibers and cell nuclei. Western blot analysis of total nuclear proteins from fetal and adult cerebral hemispheres was used to localize reactive protein bands. Preliminary results with one antibody that recognized nuclei and astrocytes stained a single band of approximately 140 kd. The monoclonal antibodies demonstrate the existence of a complex array of nuclear-associated proteins in different cell populations of the CNS and also suggests that the expression of these proteins changes substantially during development.

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354.6 AN INVESTIGATION OF NEURODEVELOPMENT IN THE CEREBELLUM USING NOVEL MARKER PEPTIDES. J. Hempstead*, J. R. Slemmon*, [1]R. W. Blacher* and J. I. Morgan* (SPON: W. D. Horst). Dept. Physiol. Chem. & Pharm., Roche Inst. of Molec. Biol., Roche Research Center, Nutley, NJ 07110, [1]Dept. Protein Chemistry, Applied Biosystems, Inc., Foster City, CA. 94404.

Three peptides, cerebellin, des-Ser[1]-cerebellin and des-Ser-Gly-cerebellin have been isolated from both rat and mouse cerebellum. The peptides have been sequenced and synthesized and sequence-directed antibodies raised against them in rabbits. Both direct chemical analysis and radioimmunoassay show the cerebellins to be at least 20 to 50 times more abundant in cerebellum than any other brain region, the olfactory bulb being the only significant extracerebellar source of the peptides. The cerebellins have been localized to the Purkinje cells of both rat and mouse cerebellum by immunocytochemistry, for which they are markers.

During development, the cerebellins may be first detected 4 to 5 days after birth in both cerebellum and olfactory bulbs. Peptide levels subsequently rise to a maximum in both structures at about the twenty-fifth day post partum. Thereafter, the cerebellin concentrations in cerebellum decline to a stable adult value. This time course of appearance of cerebellins is mirrored in the immunochtochemical picture of cerebellin expression in developing Purkinje cells. Based upon this analysis we have proposed that the initial expression of the cerebellin gene is triggered by the interaction of climbing fibres with immature Purkinje cells. The amplification of cerebellin biosynthesis is suggested to be mediated by granule cells via parallel fibre synapses. the fall of cerebellin levels may be linked to the remodeling of the Purkinje cell dendritic arbors with a concomitant loss of parallel fibre synapses..

Evidence to support the above model of the developmental regulation of cerebellin expression has come from studies involving mutant mice. Thus mutants which have reduced granule cell numbers such as Reeler and Weaver have large deficits in cerebellin levels. This suggests some direct relationship between granule cell numbers and cerebellin levels. The immunocytochemical analysis of these same granule cell-deficient mutants shows cerebellin immunoreactivity to be concentrated in Purkinje cells associated with groups of surviving granule cells, lending further support to the notion of transcellular regulation of cerebellin metabolism. The model is being further tested using lesioning and in vitro organotypic cultures of cerebellum.

354.7 PEPTIDE MARKERS OF NEURONAL DEVELOPMENT IN MAMMALS. R.Ziai* and J.I.Morgan* (SPON.R.Chizzonite). Dept.Physiol.Chem.,Roche Inst. Mol.Biol.,Roche Res.Center,Nutley,NJ 07110.

In an effort to isolate peptides useful as markers of neural development in mammals,extracts of various rat brain regions at different stages of development were analyzed by reversed phase HPLC.Two developmentally regulated peptides were detected by comparing the chromatograms obtained from neonatal and young adult brains.A neonatal brain-specific peptide (NSP) was present in the brains of embryonic and newborn rats at approximately 40-50µg/g tissue.However,its concentration fell to 3-5µg/g tissue between days 4 and 21 post partum .NSP is at least 10-fold more abundant in the neonatal brain than any other tissue examined.This peptide is present in,and actively synthesized by cultured rat neuroblastoma cells.It could not be demonstrated in astrocytoma cells, indicating that the peptide is likely to be of neuronal origin. NSP has been purified to homogeneity and interestingly,has a primary amino acid sequence that is about 80% homologous with a series of peptides(thymosins) synthesized in immunocompetent tissues: the thymosins have been suggested to be immune regulators.Antibodies to the thymosin peptides cross-react with NSP in western blots.However,NSP was not present in the immunocompetent tissues examined and no thymosins exhibited a temporal modulation comparable to NSP.

A second develòpmentally controlled neuropeptide ,designated PEP-19,was purified to homogeneity and was shown to have a restricted distribution in the adult rat brain.It was predominantly present in the adult rat cerebellum and in lower concentration in the olfactory bulb.It was undetectable in the cortex or brain stem and several other non-neural tissues examined.The concentration of PEP-I9 in the cerebellum increased by approximately sevenfold from day 2 to day 12 post partum and remained steady thereafter.Preliminary investigations have shown that the peptide has 60 amino acid residues with a molecular weight of 8 kilodaltons on SDS-PAGE.In view of the maturation profile of PEP-19,this peptide may prove to be a marker for granule cells.The sequencing of this putative peptide marker is in hand.

354.8 TRANSFERRIN PROTECTS RETINAL NEURONS FROM GLUTAMATE TOXICITY AT EARLY STAGES OF DEVELOPMENT. Gail D. Zeevalk, Carlos H. Zambrano and Arnold G. Hyndman, Rutgers Univ. Dept.Biol.Sci. Piscataway, N.J. 08854

Glutamate toxicity in CNS neurons is thought to be the result of excitotoxic interactions with glutamate receptors. Our in vitro work with cultures of purified retinal neurons from 8 day chick embryos (E8) using either glutamate or glutamate agonists or antagonists indicates that glutamate toxicity in these retinal neurons occurs via a similar phenomena. Recently, we found that this toxic response can be modified by the culture environment (ARVO,'84). Retinal neurons grown in basal medium containing insulin (Ø.83uM) and catalase (5.6U/ml)(CI) are sensitive to glutamate exposure when assayed at 72h in vitro (C3), while those grown in the defined medium supplement N1, containing insulin, transferrin (62.5nM), progesterone (36nM), putrescine (100uM) and selenium (30nM) are glutamate resistant. In view of recent reports indicating that transferrin may play a broader role in cellular development and physiology than previously thought, we investigated whether transferrin may be involved in protecting neurons from glutamate toxicity.

When transferrin was included in CI medium, survival of E8-C3 retinal neurons after exposure for 24h to 5mM glutamate was 86% of control (vs. 50% when grown in CI). The protective effect of transferrin was examined for transferrin concentrations ranging from Ø to 312.5nM. Protection exhibited dose response characteristics with significant protection beginning at 62.5nM transferrin. While overall survival was slightly greater in transferrin containing medium, the difference in response to glutamate was not due to differential survival since transferrin protection could be seen when cells were grown for 48h in CI medium and transferrin added 20min prior to glutamate treatment (77% of control). Conversely, when cells were grown in transferrin containing medium and switched at 48h to medium lacking transferrin, protection was still seen after glutamate exposure (90% of control). The ability of transferrin to protect retinal neurons from glutamate toxicity appears to be linked developmentally, since cultures from older retinal neurons (E11-C3) were not protected. We are currently investigating the role transferrin plays in normal neuronal development.

354.9 TRANSFERRIN BINDS TO EMBRYONIC RETINAL NEURONS AND ALTERS GLUCOSE METABOLISM. A.G. HYNDMAN & G.D. Zeevalk, Rutgers Univ. Dept. Biol. Sci., Piscataway, N.J. 08854

Recent evidence from several laboratories has implicated transferrin as an important factor in regulating neural differentiation. We have found that transferrin can protect some retinal neurons from glutamate toxicity (Zeevalk, et al., Neurosci. Abs.,1985) In this study we begin to examine transferrin's role in cellular physiology, using monolayer cultures of 8 day embryonic neural retina from chick.

Utilizing immunohistochemical techniques we have found that transferrin binds to neurons, but not glia. Transferrin binding was visualized in mixed cultures of neurons and glia and in purified neuronal cultures by exposure of the culture to transferrin followed by FITC-anti-transferrin anti-body or by the peroxidase anti-peroxidase method. Preliminary observations indicate that transferrin binding is most concentrated on the cell soma.

We also examined the effects of transferrin on glucose uptake in purified neuronal cultures. Cultures were maintained for 72h prior to the assay in basal medium supplemented with glucose (28mM), insulin (0.83mM) and catalase (5.6 U/ml) (CI) or CI plus transferrin (62.5nM)(CIT). The non-metabolized analog 3-O-methyl-glucose was used to measure glucose uptake. Following a 30min incubation in low glucose medium, cultures were incubated in various amounts of cold ligand plus 14C labeled 3-O-methyl glucose for 10min in the presence or absence of transferrin. The amount of label uptake was determined in the presence of four concentrations of cold ligand 8mM, 10.5mM, 15.5mM & 25.5mM. In cultures maintained in CI, uptake increased linearly throughout the concentration range. Uptake was 9.2, 22,6, 47.3, 88.9 fmol/cell/min respectively. Cultures grown in CIT showed uptake 1.5 to 4 times greater than cultures grown in CI. When transferrin was added to cultures maintained in CI, 30min before the assay, there was an 1.6 fold increase in uptake compared to controls. Transferrin induced enhancement of glucose uptake may be developmentally linked since uptake in cultures from older retinal neurons (11 day embryonic retina) was not altered by transferrin. This study suggests that one mechanism by which transferrin may regulate neuronal differentiation or sensitivity to glutamate is by altering glucose metabolism.

354.10 DEVELOPMENTAL CHANGES IN NOREPINEPHRINE-INDUCED THERMOGENESIS IN WEANLING RATS. E. Thiels* and J. R. Alberts* (SPON: R. D. Devoe). Dept. of Psychology, Indiana University, Bloomington, IN 47405.

Brown adipose tissue (BAT) is the principal site of nonshivering thermogenesis in cold-acclimated adult rats (Foster and Frydman, Can. J. Physiol. Pharmacol., 56:110, 1978) , and appears to be the effector of increased heat production following a single meal (Glick, Teague and Bray, Science, 213:1125, 1981), as well as during chronic overfeeding (Rothwell and Stock, Nature, 281:31, 1979). Both cold- and diet-induced activation of BAT are mediated by the release of norepinephrine (NE) from sympathetic terminals innervating brown adipocytes (Rothwell and Stock, Can. J. Physiol. Pharmacol., 58:842, 1980). In the present study, we investigated the thermogenic capacity of BAT in weanling-aged rats, for which a thermal and food-efficiency regulatory system independent of maternal resources becomes increasingly important.

Pairs of 15- and 20-day old pups were placed into incubators maintained at 29°or 28°C, respectively, and given a 15-min adaptation period before receiving an i.p. injection of one dose of l-NE (0.1, 0.2, 0.4, 0.8, 1.6, or 3.2 mg/kg), or an equivalent volume of normal saline. Five min before and at four 20-min intervals following injection, interscapular BAT (IBAT) temperature was measured with a thermocouple placed on the overlying skin. In general, dosages above 0.2 mg/kg produced a significant elevation in IBAT temperature relative to both pre-injection temperatures and temperatures of control pups. These changes developed rapidly and reached maximum within 60-min post-injection in both age groups. Maximal temperature as well as the magnitude of the temperature increment increased with dosage in pups of both ages, with the exception of a significant decrease of IBAT temperature in 15-day-olds receiving the highest dosage. Throughout the effective dose range, younger pups displayed greater responsiveness to NE than did older pups: dose-dependent temperature maxima were 1°-2°C higher and temperature increments 2-3 times larger in 15-day-olds compared to 20-day-olds. These results show that sympathetic activation of BAT has powerful thermogenic effects in weanling-aged rats, and that the calorigenic potential of this source of heat production decreases as additional thermoregulatory mechanisms emerge.

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354.11 SEROTONERGIC AGONIST AND ANTAGONIST EFFECTS ON MOUTHING AND INGESTION INDEPENDENT OF THE DAM IN 3-4 AND 10-11 DAY OLD RAT PUPS. E.K. Enters* and L.P. Spear. Dept. of Psychology and Center for Neurobehavioral Sciences, State Univ. of New York at Binghamton, Binghamton, NY 13901

Serotonergic antagonists have been shown to block suckling in 3-4 and 7-8 day old, but not older, rat pups, presumably by interfering with the mouthing and licking that precedes attachment (Spear & Ristine, 1982; Ristine & Spear, 1984). Serotonergic antagonists have also been observed to attenuate mouthing induced by the serotonergic agonist quipazine, or by intraoral milk infusions, in 3-4 day old deprived rat pups (Caza & Spear, 1981; Ristine & Spear, 1985). If the blockade in suckling induced by serotonergic antagonists during approximately the first postnatal week is related to a drug-induced disruption of the mouthing component of attachment, it might be expected that serotonergic manipulations would have less pronounced influences on mouthing behavior during the second postnatal week, an age when these drugs do not affect suckling. The present study compared the effects of serotonergic agonists and antagonists on mouthing in 3-4 and 10-11 day old pups in an independent ingestion paradigm. Because of recent controversy regarding the relationship between mouthing and amount ingested in this paradigm, weight gain was also assessed.

Male and female, 3-4 and 10-11 day old offspring of Sprague Dawley derived breeding pairs were used. Half of the pups were deprived of food and the dam for 24 hrs. prior to testing; the remaining pups were non-deprived. Pups were injected subcutaneously with either a 0 (0.9% saline), 1, 2.5, 5, or 10 mg/kg dose of the serotonergic agonist quipazine, or of the antagonist methysergide. Ten min. later, pups were placed in an apparatus without milk, where baseline behaviors were observed for 5 min. Pups were then placed for 10 min. in a test apparatus containing Half and Half puddled on a terrycloth floor to allow ingestion, and their behavior and post-test body weights were recorded. As expected, deprivation increased both mouthing and ingestion, and the presence of milk increased mouthing. In 3-4 day old rat pups, quipazine increased, and methysergide decreased, mouthing. At 10-11 days postnatally, both quipazine and methysergide appeared to slightly attenuate milk-induced mouthing. Thus, it appears that serotonergic manipulations may influence mouthing differently in 3-4 and 10-11 day old rat pups. The amount ingested was not observed to be associated with amount of mouthing in 3-4 day old pups, with both quipazine and methysergide decreasing ingestion at this age. At 10-11 days postnatally, decreases in ingestion were closely associated with drug-induced attenuations in mouthing.

354.12 BEHAVORIAL RESPONSES TO D-1 AND D-2 DOPAMINE AGONISTS IN NEONATALLY-6-HYDROXYDOPAMINE (6-OHDA) TREATED RATS. G.R. Breese, A. Baumeister*, T.C. Napier, G.D. Frye, G. Duncan* and R.A. Mueller. UNC Sch. of Med., Chapel Hill, NC 27514 and Texas A&M Coll. of Med., College Station, TX 77840.

In an earlier study (JPET, 231:343, 1984), it was found that L-DOPA-induced self-biting in neonatally-6-OHDA-treated rats was antagonized by cis-flupentixol, a compound with actions on D-1 and D-2 dopamine receptor sites. Since the D-2 dopamine antagonist, haloperidol, was less effective in reducing the incidence of self-biting than cis-flupentixol, it seemed possible that a subpopulation of dopamine receptors is being selectively altered in neonatally-6-OHDA-treated rats. To explore this hypothesis, behavioral responses after administration of the D-2 agonist, LY-171555, or the D-1 agonist, SKF-38393, were evaluated in neonatally-6-OHDA-treated rats. Behaviors observed in neonatally-6-OHDA-treated rats after the D-1 agonist included self-biting, "taffy pulling", licking, sniffing, rearing, paw treading, head nodding, digging and grooming. SKF-38393 produced no significant behavioral changes in untreated rats indicating that the dopamine receptors being stimulated were functionally supersensitive in the neonatally-6-OHDA treated rats. In spite of this functional supersensitivity to the D-1 agonist, $^3$H-SCH-23390 binding was not altered in striatum. Many of the behaviors observed after SKF-38393 were also observed after LY-171555 administration. Behavioral responses to D-1 and D-2 agonists did not show the same profile in adult- and neonatally-6-OHDA-treated rats, providing support for the view that the age at which dopaminergic neurons are destroyed can have differing effects on motor output. The self-biting observed after L-DOPA was also induced by SKF-38393, but not LY-171555. Furthermore, the self-biting was antagonized by SCH-23390, a D-1 antagonist. There was a high correlation between the occurrence of L-DOPA induced self-biting and supersensitive locomotor responses to SKF-38393 (Fed. Proc., 43:923, 1985) in rats treated neonatally with 6-OHDA. These data support the hypothesis that neonatally-6-OHDA treated rats have functionally supersensitive D-1 dopamine receptors which are critical for self-biting and which can contribute to locomotion as well as other behavioral responses induced by agonists such as L-DOPA or apomorphine after this treatment. (Supported by HD-03110, HL-31424, and MH-36294).

354.13 PRENATAL EXPOSURE TO NICOTINE INDUCES ACUTE AND PERSISTENT CHANGES IN RAT BRAIN CATECHOLAMINES AND THEIR METABOLITES.

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Prenatal exposure to nicotine can result in behavioral dysfunctions both in laboratory animals and in man. We recently found that the drug also affects the gonadal axis of the male rat fetus and a sexually dimorphic behavior (Lichtensteiger et al., Monogr. neural Sci., Vol. 9, 213, 1983; Pharmacol. Biochem. Behav., in press). The neurochemical basis of these functional alterations is not known. We investigated possible prenatal effects of nicotine on central catecholamine and serotonin systems. Fetal or postnatal rat forebrain was assayed for catecholamines by a thinlayer radioenzymatic method and for metabolites of catecholamines and serotonin by HPLC-EC. The study was conducted mainly on Long Evans (LE) rats; some differences were noted between LE and Sprague Dawley fetuses.

Amine metabolite concentrations rise slowly from gestational day (GD) 17 - except for an elevation at birth, with similar values in male and female fetuses. In both sexes, a single injection of nicotine (1 mg/kg s.c., 30 min) given to the pregnant dam at GD 21, increased DA, DOPAC, HVA and 5-HIAA in fetal forebrain. On postnatal day (PN) 6, the drug (0.3 or 1 mg/kg) affected MOPEG and the DA metabolites but not 5-HIAA. For chronic prenatal administration of nicotine, pregnant LE rats were implanted s.c. at GD 12 with an osmotic minipump (Alzet) which delivered nicotine at a rate of 0.25 mg/kg x hr for one week (see ref.). When checked at GD 18, the effects of this treatment differed from the acute condition; MOPEG was now increased in male and female fetuses. The metabolic pattern of the pregnant dam was different from that of her fetuses. At postnatal stages, persistent alterations in catecholamine metabolites were seen after prenatal drug exposure. The metabolite pattern of nicotine-treated offspring changed from GD 18 (when the drug was still present) through PN 15 until adulthood and differed between sexes. Adult male offspring exhibited reduced levels of MOPEG, DOPAC and HVA in forebrain, while their female littermates showed but a reduction of HVA. An analysis of cross-fostering data revealed that the changes were due to prenatal influences.

These observations demonstrate 1) that central monoamine systems of the fetus are responsive to acute administration of nicotine and 2) that prolonged prenatal exposure to the drug can result in persistent alterations in catecholamine metabolites especially in the male.

354.14 COMPARISON OF PRENATAL AND POSTNATAL NICOTINE ADMINISTRATION ON NEUROMUSCULAR MATURATION, K. J. Rose* and F. L. Strand. Biology Department, New York University, Washington Square, New York, NY 10003.

A study was undertaken to investigate whether nicotine administration to pregnant rats and/or their offspring influences the character and maturation of the neonatal neuromuscular system. The in situ extensor digitorum longus (EDL) muscle and peroneal nerve complex was used as a model. Pregnant females received either nicotine tartrate (0.5 mg/kg) or saline as control twice daily i.p. from day 3 to day 18 of gestation, then once daily until parturition. Pups were injected with either nicotine tartrate (0.05 mg/kg/day s.c.), or saline as control. All pups were tested on the 14th and 15th day after birth. Animal subgroups consisted of 4-7 pups.

Pups treated with nicotine during prenatal and postnatal life as compared to those pups treated with saline throughout, showed no change in mean motor unit size or number, nor was there a change in tetanic or twitch contraction amplitude. However, the durations of twitch contraction and half-relaxation were decreased, indicating a more rapid twitch. Tetanic contraction time increased and half-relaxation time remained constant, indicating an improved ability to sustain tetanus. No nicotine-induced changes occurred in post-rest or post-tetanic potentiation.

Pups treated with nicotine during both prenatal and postnatal life showed a significant decrease in tetanic half-relaxation time when compared to pups that received nicotine prenatally only. Other contractile parameters were essentially the same as in pups exposed to nicotine throughout. Thus, twitch times for pups treated with nicotine postnatally only had faster twitches than saline controls.

Offspring receiving nicotine pre- and postnatally had a significantly shorter twitch half-relaxation time than pups receiving nicotine postnatally only. Similarly, tetanic contraction time is longer in those animals that received nicotine throughout. Postnatal nicotine administration did not significantly alter contractile parameters when compared to saline controls.

During normal development, in those muscles destined to become predominantly fast muscle, such as the EDL, contractile parameters are initially the same as those of slow fibers. Nicotine administration during prenatal life appears to accelerate this maturation process, whereas postnatal administration has relatively little effect as seen two weeks after birth. The possibility that such changes may be mediated through nicotine-evoked ACTH release is being investigated.

We thank The Council for Tobacco Research for their support of this study.

354.15 THE EFFECT OF PRENATAL EXPOSURE TO PHENCYCLIDINE ON THE POSTNATAL CONCENTRATION OF ACETYLCHOLINE AND CHOLINE IN THE RAT.

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Phencyclidine (PCP) has been demonstrated to have a biphasic effect on dopamine (DA) release in the caudate nucleus. PCP is thought to be an indirectly acting DA agonist at low doses, however it has been shown that at high doses PCP inhibits DA release. This pharmacological perturbation of DA release by PCP has also been shown to effect the endogenous levels and rate of synthesis of acetylcholine (ACh) in various brain regions. In this study we have examined the effect of pre- and postnatal exposure to PCP on endogenous and newly synthesized ACh in the 21 and 35 day old rat pup.

Female Sprague-Dawley rats were injected with PCP (0.5 mg/kg s.c.) throughout gestation. The subsequent litters of rat pups were treated with PCP daily (0.5 mg/kg s.c.) either from 0-21 days postnatal or from 0-35 days postnatal. Rat pups were sacrificed by microwave irradiation after a pulse injection of deuterium-labelled choline (20 umol/kg i.v.). The concentrations of endogenous and deuterium-labelled ACh and choline were measured using a GC-MS assay.

The concentration of both endogenous and deuterium-labelled ACh was increased in the 21 day old rat pups that were exposed to PCP pre- and postnatally. A similar response was seen in the frontal cortex. After 35 days, the effect of chronic pre- and postnatal PCP treatment on endogenous ACh levels was no longer significantly different from control values. However synthesis, as measured by deuterium-labelled ACh, was significantly increased over control values.

These data suggest that pre- and postnatal exposure to PCP markedly effects the cholinergic system in the caudate nucleus and frontal cortex of the developing rat pup.

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354.16 ALTERED NOCICEPTIVE RESPONSIVENESS IN ADULT RATS FROM PRENATAL EXPOSURE TO MORPHINE OR CLONIDINE. E. LaCrosse*, B. Consoliver* and B. Culver Neurosci. Prog., School of Pharm., Univ. Wyoming, Laramie, WY 82071

Previous studies in our laboratory demonstrated an antinociceptive effect produced by infusion of adult animals with the alpha-2 adrenergic agonist clonidine. Interestingly, this effect was seen in rats when tested not only during the 1 week infusion period, but also for 2 weeks following discontinuation of the drug. Enduring antinociceptive reactions have also been reported for adult offspring of rats given opiates during pregnancy. Experiments in this study were designed to extend these findings to compare the effects of prenatal exposure of rats to clonidine and to morphine.

Sixteen pregnant female rats were assigned to one of 4 groups (4 rats/group) on day 14 of gestation. Rats in one group (CL) received clonidine infusions (2µg/hr) from s.c. implanted osmotic pumps. Paired control rats (CO) were subjected to identical surgical procedures. Another group of rats (MS) were implanted with sialastic morphine pellets (100 mg. morphine $SO_4$/pellet) on gestation days 14 (1 pellet) and 17 (2 pellets). A paired control group of rats (LA) were implanted with sialastic lactose pellets on the same days. At 2 mo of age litters were grouped by sex and CL and MS groups were paired for study with animals in the CO and LA, respectively, control groups. The animals were studied for 4 consecutive days using tail flick (TF) and paw lick (PL) tests. Latency to the TF response was significantly elevated in CL groups, both male and female, compared to CO groups. MS groups also showed an increased latency to response in TF compared to LA groups. There were no differences found with the PL test but this may have been due, at least in part, to the high variability of this data.

The findings of similar effects from prenatal exposure to clonidine or morphine suggest the drugs share a common action resulting in altered nociceptive responsiveness in the adult. Since both clonidine and the opiates have been shown to inhibit firing of noradrenergic neurons in the locus coeruleus, it is tempting to speculate that the underlying mechanism of the observed effect involves an interaction at presynaptic noradrenergic sites.

354.17 FAILURE OF NEONATAL RATS TO DEVELOP TOLERANCE AND WITHDRAWAL SYMPTOMS FOLLOWING REPEATED EXPOSURE TO MORPHINE. C. P. Cramer and M. S. Fanselow*. Department of Psychology, Dartmouth College, Hanover, NH 03755.

Adult rats exposed to moderate doses of opiates are initially analgesic; however, after additional exposures they become tolerant, as indicated by a reduction or elimination of analgesia. Furthermore, when tolerant adults are challenged with opiate antagonists such as naloxone, they show signs of withdrawal, such as "wet dog shakes" and diarhhea. In the present studies, we explored the development of tolerance to opiates in neonatal rats. Our findings suggest that neonates do not develop either tolerance or withdrawal symptoms, even after 21 days of repeated morphine exposure.

Beginning on the day after birth, infant rats were injected daily with either morphine sulfate (10 mg/kg, i.p.) or vehicle (saline). Twenty minutes after injection, they were tested by manually supporting them above a 52°C hotplate and allowing the bottom of one hindpaw to come in contact with the plate. Latency to lift the paw was used as a measure of analgesia. Saline-injected pups consistently lifted their paws with a latency of 2-4 seconds; performance did not change with age. The mean latency to pawlift among morphine-treated animals was at or near the 15 sec. cut-off latency throughout the 21 day exposure period. To directly compare analgesic responses, on Day 22 both morphine-treated rats and previously saline-injected controls received 5 mg/kg doses of morphine (to avoid ceiling effects). Both groups showed an equivalent and profound analgesic response to the drug. On Day 23, all pups were challenged with naloxone. Even those animals that had received 22 previous exposures to morphine did not show any evidence of withdrawal symptoms.

We obtained similar results using a lower dose of morphine (5 mg/kg) throughout the exposure period. In addition, another group of rats received daily exposure to either morphine (5 mg/kg) or saline beginning at 5 days of age. Those treated with morphine grew at normal rates but continued to be highly analgesic from Days 5 to 20 postpartum, indicating that our previous results were not due to retarded growth.

Thus, neonatal rats failed to exhibit tolerance and abstinence-related behaviors, despite extensive experience with low and moderate doses of morphine. These data suggest that phenomena associated with prenatally developed tolerance and withdrawal should be re-examined.

354.18 NEONATAL MORPHINE EXPOSURE ALTERS ADULT MOTOR BEHAVIOR IN RATS. G.E. Handelmann, Lab. of Developmental Neurobiology, NICHD-NIH, Bethesda, MD 20205.

Exposure to opiates during development in humans has a detrimental effect on motor behavior. Children exposed to narcotic drugs in utero show retarded motor development, perform poorly on perceptual motor tasks, and have poor fine-motor coordination (e.g. Wilson et al., J. Ped. 98: 716, 1981; Strauss et al., J. Ped. 89: 842, 1976). These findings suggest that exposure to opiates adversely affects development of motor regions of the brain. We showed previously that, in the rat, neonatal exposure to morphine increased the number of mu opiate receptors in the adult basal ganglia (Handelmann and Quirion, Eur. J. Pharm. 94: 357, 1983). The increase in receptors was correlated with a decrease in 2-deoxy-glucose uptake in this and other motor areas, suggesting decreased activity (Handelmann and Dow-Edwards, Peptides, in press). The present experiments were performed to determine whether these changes in function in brain motor areas are of behavioral significance to the rat.

Neonatal albino rats were injected subcutaneously with either morphine (1 µg/ pup in phosphate-buffered saline [PBS]) or PBS on days 1-7 after birth. When the rats were at least 60 days old, they were tested on several measures of rat motor behavior: motor coordination on a rotor rod, gait, and spontaneous activity in an open field. The rats treated as neonates with morphine were unable to remain on the rotating rod as long as the PBS-treated rats (2.7 ± 0.4 vs. 5.8 ± 1.0 secs, $p<.05$). In addition, their gait while walking in a straight alley was different from the controls. Their stride length was longer, and their foot placement relative to the direction of the track was unusual: they tended to walk with their feet turned out in a "duck-footed" gait. Finally, the rats treated as neonates with morphine were no more active in an open field than were the controls, but they were more likely to enter the center portions of the field.

These results indicate that neonatal exposure to morphine in the rat has long-lasting effects on motor behavior. Some of the behavioral effects are consistent with abnormal function of the basal ganglia, suggesting that the opiate receptor and metabolic changes in this region due to early morphine exposure are of physiological significance to the animal.

## RETINA IV

355.1 GABA AND GAD IMMUNOREACTIVITY IN PHOTORECEPTOR TERMINALS OF THE RHESUS MONKEY RETINA: ELECTRON MICROSCOPIC ANALYSIS. Y. Nishimura*, M.L. Schwartz and P. Rakic. Section of Neuroanatomy, Yale Univ. Sch. Med., New Haven, CT 06510.

Our previous light microscopic immunocytochemical analysis of the monkey retina (Nishimura, Schwartz and Rakic, 1985) consistently revealed immunoreactivity to gamma amino butyric acid (GABA) and L-glutamic acid decarboxylase (GAD) that was situtated in a narrow band within the outer half of the outer plexiform layer (OPL). In the present study, we examined the localization and possible sources of this immunoreactivity at the electron microscopic level. Retinas from 16 rhesus monkeys (Macaca mulatta) were fixed by perfusion with various combinations of aldehydes in phosphate buffered saline (PBS). Vibratome sections, 50 um thick, were cut through the perifoveal region of the retina and reacted with either anti-GABA serum (Immunonuclear Corp.) or anti-GAD serum (Ortel et al., Neurosci. 6: 2689, 1980) using the avidin-biotin-peroxidase conjugation method. Reacted retinas were postfixed, embedded, and prepared for EM analysis. We also conducted several control experiments including incubation of retinas with normal rabbit serum (for GABA) or normal sheep serum (for GAD) in place of the primary antiserum, as well as incubation in anti-GABA serum that had been preabsorbed with GABA. No immunoreactivity was seen in the OPL in any of these controls.

Electron microscopic examination of the OPL revealed the presence of a considerable number of GABAergic terminals in retinas reacted against either anti-GABA or anti-GAD sera. Dense immunoreactivity was found in some terminal profiles while other terminals in the same section were completely devoid of GABA or GAD labeling. Frequently, immunoreactivity was localized in the subclass of synaptic terminals having the size and morphology characteristic of rod spherules including the presence of electron dense synaptic ribbons surrounded by synaptic vesicles. In some sections of the perifoveal region, as many as 25% (n=312) of these profiles contained immunoreactive product. In some instances, immunopositive profiles could be followed in serial sections from the terminal bouton through the inner portion of their connecting fibers. However, the perikarya of photoreceptor cells were invariably unstained. Moreover, only a few cone terminals identified by their wide based pedicles, multiple indentations, and multiple synaptic junctions were positive. The pattern of GABA and GAD immunoreactivity that we have observed in a large number of monkey retinas suggests that a class of photoreceptors, primarily rods, contains the inhibitory neurotransmitter GABA. Thus, our results indicate that, in addition to the cells within the inner nuclear and ganglion cell layers previously reported to be GABAergic, some photoreceptors in the primate retina also may use GABA as a neurotransmitter. Supported by grant EY02593.

355.2 GABA-LIKE IMMUNOREACTIVITY IN HORIZONTAL CELLS OF THE CAT RETINA J. Bolz* and B.A. McGuire, Laboratory of Neurobiology, The Rockefeller University, New York, NY 10021

The identification of presumed GABAergic neurons in the retina was originally based on the autoradiographic localization of neurons that accumulate tritiated GABA. In lower vertebrates, horizontal, amacrine, and displaced amacrine cell populations accumulated GABA. In mammals, amacrines, displaced amacrines, and interplexiform cells showed GABA uptake, but the horizontal cells did not. A similar pattern of labeling has been reported in more recent immunohistochemical studies with antibodies against glutamic acid decarboxylase (GAD), the rate-limiting enzyme of GABA synthesis.

In the present study we have attempted to localize putative GABAergic neurons in the cat retina using an antiserum directed against GABA. Similar to the results from the uptake experiments, we found GABA-positive amacrines, displaced amacrines and interplexiform cells. However, we also found GABA-like immunoreactivity in horizontal cells. Specificity of the staining was assessed by preabsorbtion of the primary antiserum with synthetic GABA. This procedure blocked the immunolabeling of all cell classes. The morphology and distribution of the horizontal cells was studied in tangential sections processed for GABA-like immunoreactivity with the peroxidase-anti-peroxidase technique. In the axonless A-type horizontal cells reaction product was found in somata and dendrites, whereas in B-type horizontal cells the label was seen in the somata and axon terminal systems but not in the dendrites. We found the same number of labeled A- and B-type horizontal cells as Wässle et al. in their reduced silver and Nissl stained material (Proc. R. Soc. Lond. B 203: 269-291,1978), indicating that all horizontal cells show GABA-like immunoreactivity.

To obtain further support for the suggestion that GABA may be the transmitter for horizontal cells, we have looked for GAD-like immunoreactivity in retinal sections using an antibody kindly provided to us by Dr. W. Oertel. We always found immunoreactive amacrine cells, whereas horizontal cells were only occasionally labeled. The reason for this inconsistency is unclear. It remains to be determined whether cat retinal horizontal cells do indeed lack an active uptake mechanism for GABA or whether the uptake is masked, for example, by the prominent uptake of GABA into Muller cells. Nevertheless, the consistent labeling of horizontal cells by the GABA antibody does suggest that GABA might be the transmitter of horizontal cells in the cat retina, and this would be in accordance with the inhibitory roles attributed to these cells such as surround inhibition.

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355.3 DISTRIBUTIONS OF ASPARTATE AMINOTRANSFERASE AND PHOSPHATE-DEPENDENT GLUTAMINASE ACTIVITIES AMONG RETINAL LAYERS.
C. David Ross, Marc Bowers*, and Donald A. Godfrey (SPON: J.E. Norvell). Department of Physiology, Oral Roberts Univ., Tulsa, OK 74171

The total activity of aspartate aminotransferase (tAAT= combined activities of mitochondrial (mAAT) and cytosolic (cAAT) isoenzymes) was determined in retinal layers from rat, guinea pig, turtle, and frog. In addition, the activities of the cAAT isoenzyme (Parli, et al., Soc. Neurosci. Abstr., this volume) and phosphate-dependent glutaminase (PDG) were determined in retinal layers from rat and guinea pig. tAAT activity was highest in the photoreceptor inner segments (IS) in all 4 species, was high in the outer plexiform layer of rat and, especially, frog, and was relatively high in the inner plexiform layer of rat and frog. tAAT activities in layers deep to IS were low in guinea pig and turtle. The magnitude of cAAT activity varied about two-fold across layers in guinea pig and rat while the mAAT activity (taken as = tAAT - cAAT activity) varied about 10-fold in guinea pig and about 5-fold in rat. cAAT activity relative to tAAT activity had its lowest percentage in IS (15-30%) and highest in outer nuclear layer (60-75%), with intermediate values in the other retinal layers.

PDG activity was much higher in IS than in any other layer examined in both rat and guinea pig.

(Supported by NIH grant EY 03838 and by ORU intramural funds.)

355.4 MONOCLONAL ANTIBODIES AGAINST GOLDFISH RETINAL CELLS.
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We have used monoclonal antibodies as immunological probes to study the functional organization and development of the vertebrate retina. By immunizing mice with dissociated goldfish retinal cells (using the methods of Sarthy and Lam, Brain Res. 176:208, 1979), we have produced several monoclonal antibodies that recognized discrete cell types in the goldfish retina. The antibodies obtained were screened using immunofluorescence and immunoperoxidase methods.

One of the monoclonal antibodies, MH1, labeled Müller(glial) cells and a population of horizontal cells of many vertebrate retinas. In some species, only Müller cells or certain horizontal cells, but not both, were stained. We have attemped to characterize the antigen recognized by MH1 by immunoblotting. When purified vimentin and desmin were run with a goldfish retinal protein preparation on SDS polyacrylamide gels and transfered to nitrocellulose paper, antibody MH1 selectively labeled a band of goldfish retinal protein which has a molecular weight very similar to the mammalian vimentin protein. However, MH1 did not stain the mammalian vimentin protein. Conversely an antibody against mammalian vimentin also did not bind to this band of goldfish retinal protein. These studies suggest that (1) MH1 recognizes a retinal protein that shares some of the properties of mammalian vimentin, and (2) in some retinas, certain horizontal cells, which are interneurons, may possess a common antigen with glial(Müller) cells.

Additionally, we have a monoclonal antibody which only recognized Müller cells of the goldfish retina. Finally, we have produced a monoclonal antibody, GC1, which recognized some of the somas in the ganglion cell layer and their axons as well as some cells in the inner nuclear layer. These and other monoclonal antibodies are being further characterized in our laboratory. They may provide a useful tool to study the structure, function and development of vertebrate retinal cells.

Supported by the Retina Research Foundation (Houston).

355.5 **MONOCLONAL ANTIBODIES AGAINST SPECIFIC STRUCTURES IN THE RETINA OF RAT, CAT AND RABBIT.**
E.J. Tancred*, Z. Dreher* and J. Stone. School of Anatomy, University of New South Wales, Kensington, N.S.W., Australia.

Monoclonal antibodies have been developed which bind to specific structures in the retinas of several mammalian species. These antibodies have proved effective markers in the study of retinal development and structure.

The first antibody, ZD-14, binds to an antigen found in the inner and outer plexiform layers of the rat retina. It is a sensitive marker for the development of synaptic layers in the rat, demonstrating the formation of the outer plexiform layer at postnatal day (P) 8.

A second antibody, ZD-11, binds to restricted parts of Muller cells in the retina of the rat and rabbit but not cat. In the rat it binds to the inner processes and end feet whereas in the rabbit it binds to the outer processes of the Muller cells. On immunoblots from SDS-polyacrylamide gels its antigen stains a band of 45K - 66K daltons and is first expressed in the rat retina at P7.

A third antibody, ZD-1, binds to astrocytes in the nerve fibre layer of the rat, rabbit and cat, demonstrating what may be the entire extent of these cells. It also binds to restricted segments of Muller cells in the cat and rabbit, but not in the rat. ZD-1 binds in a similar manner to anti-GFAP antiserum, but is slightly more specific. It has proven effective in demonstrating developing astrocytes in the rat retina, becoming positive in the rat at about P14.

ZD-1 has also been applied successfully to wholemounts of retinas of all three species. It demonstrates that astrocytes (1) invest blood vessels extensively adapting their morphology to the shape of neighbouring vessels, (2) invest fiber bundles adapting their morphology to the orientation and size of the bundles, (3) are stellate in form where vessels and nerves are sparse, and (4) are absent from avascular areas of retina.

This latter finding is particularly clear in the rabbit retina in which the retinal circulation extends only over a 'streak' of myelinated axons extending horizontally from the optic disc. ZD-1 (and GFAP) positive astrocytes are restricted in distribution to this vascular region. The finding was confirmed by examination of the retina of the Australian possum, Trichosurus vulpecula, which is avascular, except for a few vessels at the optic disc. In this animal, ZD-1 (and GFAP) positive astrocytes could only be found at the optic disc and its margin.

355.6 QUANTITATIVE ANALYSIS OF NEUROPEPTIDE-Y CONTAINING AMACRINE CELLS IN THE TURTLE RETINA.
W.D. Eldred, E. Howard* and J.M. Polak. Dept. of Biology, Boston Univ., Boston, MA 02215; Dept. of Histochemistry, Royal Postgraduate Med. Ctr., Hammersmith Hosp., London, England W12 OHS

This study used antiserum directed against neuropeptide-Y (NPY) to selectively label amacrine cells in the turtle retina. Retinal reconstructions were performed to determine the regional densities. There were no clear differences in regional density, except for a slight increase toward the nasal region. The number of neurons ranged from 15-47 per 0.5mm squared. Cell bodies were located in the third tier of the inner nuclear layer and the cell processes were found in laminae 1, 3, and at the border of 4 and 5 within the inner plexiform layer (IPL). Processes in lamina 1 were sparse and delicate, with small synaptic boutons. Processes in lamina 3 were numerous and often coarse, with many large and small synaptic boutons. At the border of laminae 4 and 5 the processes were frequently numerous but slender, with many small synaptic boutons.

Twenty neurons from various regions of the retina were drawn in detail using a camera lucida attachment. Circular distribution statistics were used to quantitatively characterize the dendritic arborizations of these neurons. It was possible to statistically classify the cells as monopolar, bipolar, bipolar skewed, or multipolar. Although each catagory was represented, monopolar and multipolar cells were found to be more common than bipolar and bipolar skewed cells. In addition to the overall dendritic orientation for a given neuron, it was possible that there were specific orientations for the dendritic arborizations within individual lamina of the IPL. This study indicated that circular distribution statistics were effective for analyzing the morphology of individual neurons. It further suggested that a given neuropeptide may be found in more than one anatomically distinct class of amacrine cells. Supported by EY04785 to WDE.

355.7 SEROTONIN-LIKE IMMUNOREACTIVITY IN AMACRINE CELLS OF THE LIZARD RETINA. G. A. Engbretson, K. J. Anderson*, and B. -A. Battelle†. Institute for Sensory Research, Syracuse University, Syracuse, NY 13210 and †C. V. Whitney Laboratory, University of Florida, St. Augustine, FL 32086.

A population of amacrine cells in the retina of the lizard, *Uta stansburiana*, exhibits immunoreactivity to a highly specific antibody (Immunonuclear) directed against serotonin (5-HT). Cryostat sections of fixed retinas were incubated in diluted antibody and then processed by immunofluorescence or peroxidase-antiperoxidase secondary antibody techniques. The 5-HT-like immunoreactivity was localized to a population of amacrine cells with somata (≈10 μm dia.) in the inner nuclear layer just adjacent to the boundary of the inner plexiform layer (IPL). Processes emanated from the soma and branched, sending arborizations to both sublamina 1 and to sublaminae 4 and 5 of the IPL. In control experiments where the antibody was preincubated with its antigen (5-HT conjugated to bovine serum albumin) before use, immunoreactivity of the amacrine cells was blocked completely.

Golgi-stained retinas of this lizard contain amacrine cells which appear identical in size, position, and branching pattern to those cells which exhibit 5-HT-like immunoreactivity.

Preliminary analyses of acidic extracts of lizard retinas by high performance liquid chromatography and electrochemical detection revealed the sharp peak of a substance which eluted with the same retention time as 5-HT. These preliminary analyses suggest that the retina of *U. stansburiana* contains endogenous 5-HT in the amount of at least 1.5 ng/retina and that the 5-HT is confined to a population of amacrine cells.

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355.8 **SUBSTANCE P (SP) IN THE PIGEON RETINA: A QUANTITATIVE ASSESSMENT.** Kent T. Keyser* and Harvey J. Karten, Depts. of Psychiatry and Neurobiology, State University of New York, Stony Brook, NY 11794.

Biochemical and immunohistochemical studies have demonstrated the presence of SP within retinal amacrine cells in the retinae of many vertebrates, including pigeons (Karten and Brecha, 1980). The regional distribution and the variety of cell types have, however, been less clear. In particular previous studies in the pigeon have suggested that SP amacrine cells occur primarily in the peripheral retina and are either markedly diminished or absent from central retinal regions, such as the fovea and Red Field. We have examined retinal flat mounts and horizontal sections processed with monoclonal antibodies against SP (Sera-Labs) combined with avidin-biotin peroxidase staining. As suggested by Ishimoto, et al. (1981), at least two major types of SP containing amacrine cells were noted: large cells, ca. 8-9 um diameter, and a second group, ca. 6um in diameter. The larger cells stained more intensely and arborized in layer 3b. The smaller cells were lightly stained with arbors in layer 1, and occasional processes into layer 5. However, both types of cells were found in all regions of the retina, although there were some differences in total density in different regions of the retina. In representative samples of the central portion of the Red Field the total density of large and small cells was 500-800/mm$^2$ with large cells slightly more common. The combined density in the central Yellow Field was 1200-2000/mm$^2$, with large and small cells present in approximately equal numbers. In addition a third population of SP amacrine cells, possibly a variant of one of the above types, was noted in the region of the pecten oculi. This study indicates that SP amacrine cells are more numerous in all regions of the pigeon retina than previously appreciated. We suggest that the SP populations in other vertebrate retinae may also require re-evaluation.

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355.9 DEVELOPMENT OF CHOLINE ACETYLTRANSFERASE-LIKE IMMUNOREACTIVITY AND ACETYLCHOLINESTERASE IN THE CHICK RETINA. A.W. Spira, T.J. Millar, I. Ishimoto* and I.G. Morgan. Dept. of Behavioural Biology, RSBS, Australian National University, ACT, Australia, 2601.

Whereas the biochemical development of the cholinergic transmitter system of the retina has been extensively studied in the chick embryo, morphologic studies heretofore have described only the localization of acetylcholinesterase (AChE) activity. We describe here sites of potential synthesis of acetylcholine detected by the application of antisera against chicken choline acetyltransferase to cryostat-sectioned and flat-mounted retinas of chick embryos. Retinal samples were analyzed from the sixth to eighteenth days of incubation (H-H stages 30-44) and during the first week after hatching. The presence of choline acetyltransferase-like immunoreactivity (ChAT-IR) was appraised vis-a-vis AChE activity (Karnovsky-Roots method) and histologic appearances (cresyl violet staining).

ChAT-IR and AChE staining was followed through five successive steps. 1) Days 6-7. All cells lie within a single neuroblast layer (NBL). ChAT-IR and AChE were initially limited to one to two cell layers at the inner border of the NBL. ChAT-IR cells were subsequently present at some distance from the inner retinal surface and in central regions of flat-mounted retinas. AChE-stained cells then comprised the inner one-third of the NBL. 2) Days 7-8. The inner plexiform layer (IPL) forms and separates cells into a ganglion cell layer (GCL) and an inner nuclear layer (INL). A row of ChAT-IR cells bordered both sides of the IPL. AChE was present in both the GCL and INL and in a diffuse band in the IPL. 3) Days 8-11. The outer plexiform layer forms and the IPL thickens. Some cells of the GCL are temporarily "trapped" in an intra-IPL position. ChAT-IR cells were found at the innermost part of the INL and in the intra-IPL position. ChAT-IR cells were evident in all regions of flat-mounted retinas. Their processes spread laterally forming two bands in the IPL. These corresponded in position to AChE-stained laminae. AChE was present in the two separated groups of GCL cells. 4) Days 11-15. Photoreceptor cell differentiation begins with formation of their inner segments. The major period of ganglion cell degeneration occurs. ChAT-IR laminae in the IPL were further distant from their cells of origin. AChE cells in the GCL were in one stratum. 5) Days 15-early post-hatch. Photoreceptor cells develop outer segments and mature. ChAT-IR cells were added in the middle of the INL to the pre-existing reactive INL cells. AChE activity arose in two intermediate lamellae of the IPL.

ChAT-IR retinal cells thus appeared before the formation of the IPL and the onset of synaptogenesis (day 10). Changes in their appearance and prevalence was limited to an early increase in their spacing and the late addition of cells to the INL. (A.W.S. was supported by the MRC and NSERC of Canada).

355.10 EPINEPHRINE REGULATES SYNAPTIC TRANSMISSION MEDIATED BY CHOLINERGIC NEURONS OF THE RAT RETINA. *W-H. Tsai, M. S-W. Koh* & D.C. Puro.* Laboratory of Vision Research, National Eye Institute, National Institutes of Health, Bethesda, MD and Bascom Palmer Eye Institute, University of Miami, Miami, FL.

The purpose of this study was to investigate the effects of epinephrine on the function of synapses formed by cholinergic neurons derived from the rat retina. We used an experimental culture system in which striated muscle cells served as postsynaptic targets for cholinergic neurons of the embryonic retina. This culture system permitted the physiological monitoring of acetylcholine release at synapses formed by retinal neurons.

Trypsin-dissociated retinal cells from embryonic day 21 rats were co-cultured with rat striated muscle cultures. Functional retina-muscle synapses form rapidly, as determined by the presence of spontaneous synaptic potentials detected by intracellular recordings from the muscle cells. At 2 days of coculture approximately 70 % of the innervated myotubes could be demonstrated to have synaptic input that could be evoked by the microiontophoretic application of glutamate. Here, we report that epinephrine can regulate the efficacy of stimulus-evoked transmission across retina-muscle synapses.

Our electrophysiological experiments indicate that the microiontophoretic application of epinephrine had no effect on spontaneous transmission at retina-muscle synapses. However, after a 1 minute exposure to epinephrine, glutamate-evoked transmission across these synapses could be facilitated. A greater than two-fold increase in the number of evoked postsynaptic events was declared a facilitory effect. Such an effect of epinephrine on glutamate-evoked transmission was found in 80 % (12/15) of the cases examined. The facilitation by epinephrine is reversible, can be mimicked by isoproterenol (a $\beta$-agonist) and blocked by propranolol (a $\beta$-blocker). Neither the $\alpha_2$-blocker, yohimbine, nor the dopamine receptor antagonist, haloperidol, blocked this effect of epinephrine. Since epinephrine was not found to influence the membrane potential of muscle cells nor the responses of myotubes to microiontophoretically applied acetylcholine, epinephrine appeared to have mediated its effect on cholinergic transmission by affecting retinal neurons. Because our previous findings indicated that cAMP may be involved in the regulation of evoked transmission at retina-muscle synapses, the effect of epinephrine on cAMP levels was investigated. Biochemical studies demonstrated that epinephrine (5 $\mu$M) could increase cAMP levels in retinal cell cultures by more than 10-fold within one minute. The elevation of cAMP levels by epinephrine could be blocked by propranolol, but not by yohimbine nor haloperidol.

In summary, epinephrine can mediate a marked facilitory effect on stimulus-evoked cholinergic transmission in our cell culture system. This effect of epinephrine appears to be mediated via a $\beta$-receptor and may involve an increase in cAMP levels.

355.11 STARBURST-LIKE AMACRINE CELLS IN CAT RETINA ARE CHOLINERGIC. R.G. Pourcho and K. Osman* . Dept. of Anatomy, Wayne State Univ. School of Medicine, Detroit, MI 48201.

A large volume of evidence implicates acetylcholine (ACh) as a neurotransmitter in vertebrate retinas. In the rabbit, cholinergic cells are particularly well developed and exist in matching subpopulations of amacrine and displaced amacrine cells which give rise to processes ramifying in strata 2 and 4 of the inner plexiform layer (IPL) [Masland and Mills, J. Cell Biol., 83:159, 1979]. In Golgi preparations, these cells exhibit a characteristic starburst appearance (Famiglietti, Brain Res., 261: 138, 1983). Golgi studies in our laboratory have shown that the cat has a population of starburst-like amacrine and displaced amacrine cells which resemble the cholinergic cells of the rabbit although their branching pattern is relatively sparse. The somas of these cells measure 8-10 um in diameter and give rise to 1-4 primary dendrites which branch dichotomously within a narrow stratum into 3-5 generations of processes to cover a dendritic field of 300-400 um. Computer-assisted analysis showed that processes of the amacrine layer cells ramify in stratum 2 of the IPL whereas those of the displaced amacrines ramify in stratum 4. The displaced amacrines resemble the A14 amacrine cells of Kolb et. al. ( Vision Res., 21:1081, 1981). We have employed autoradiography and immunocytochemistry to determine whether these cells are cholinergic.

For autoradiography, retinas were incubated in 0.3 uM ($^3$H)choline followed by a chase in unlabeled choline with 20 mM Mg++ and 30 uM physostigmine to allow for synthesis of ($^3$H)ACh. The tissue was fixed in 3% phosphomolybdic acid (Tsuji, Neurosci. Lett., 45:151, 1984) with 2% glutaraldehyde, postfixed in osmium, dehydrated, and embedded in Epon. Small neurons on either side of the IPL were heavily labeled. Additional labeling was seen in photoreceptor outer segments and in large ganglion cells. For immunocytochemistry, retinas were fixed in 4% paraformaldehyde, rinsed in PBS, and sunk in 30% sucrose. Tissue was preincubated in 0.3% Triton X-100 prior to incubation for 24-48 h in a monoclonal antiserum against ChAT (Boehringer- Mannheim). Immunoreactivity was visualized by the peroxidase antiperoxidase technique. Staining was confined to amacrine cells and displaced amacrines with processes ramifying in strata 2 and 4, respectively, of the IPL. The density of ChAT-containing displaced amacrine cells was approximately 600 cells per mm$^2$, exceeding the amacrine layer cells by nearly 3:1. Camera lucida drawings of ChAT immunoreactive cells show the same morphological features as the Golgi preparations of A14 or starburst-like cells. We conclude that these cells are cholinergic.

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355.12 SYNAPTIC ORGANIZATION OF CHOLINERGIC NEURONS IN THE CHICKEN RETINA. I.G. Morgan, I. Ishimoto* and T.J. Millar*. Department of Behavioural Biology, RSBS, Australian National University, Canberra, ACT 2600, Australia.

We have investigated cholinergic neurons in the chicken retina, particularly their synaptic organizations, using immunohistochemical techniques with an antiserum against chicken choline acetyltransferase. Immunoreactivity was found in cells in the inner nuclear layer; cells in the ganglion cell layer; two compact fiber bands between sublaminas 1 and 2 and sublaminas 3 and 4 of the inner plexiform layer; and in scattered varicose fibers in sublaminas 4 and 5 of the inner plexiform layer.

Immunoreactive cells in the inner nuclear layer comprised two populations. One population was located at the border of inner nuclear and inner plexiform layers, and dendrites from these cells produced the compact fiber band between sublaminas 1 and 2. The other population was located 3-4 cell layers from the border. By using selective lesioning of retinas with cholinergic neurotoxin AF64A, it was established that dendrites from this population of cells were the main source of varicose fibers in sublaminas 4 and 5. The immunoreactive cells in the ganglion cell layer were displaced amacrine cells and their dendrites gave rise to the compact fiber band between sublaminas 3 and 4.

Although quantitative evaluation is still preliminary, the most striking ultrastructural feature was the large number of cholinergic/cholinergic synapses. This was apparent in both compact immunoreactive fiber bands. Other input to cholinergic processes was from bipolar cells. Cholinergic outputs onto ganglion cells and non-labelled amacrine cells were seen in both compact bands, whereas output to bipolar cells was only seen in the compact immunoreactive fiber band between sublaminas 1 and 2 of the inner plexiform layer.

We suggest, because of the large number of cholinergic/cholinergic synapses, that cholinergic cells have directional sensitive functions in the retina.

355.13 PATCH-CLAMP RECORDINGS FROM GANGLION CELLS ISOLATED FROM GOLDFISH RETINA: RESPONSES TO ACETYLCHOLINE S. Naghshineh*, M.J. Fain* and G.L. Fain, Department of Ophthalmology, Jules Stein Eye Institute, UCLA, Los Angeles CA 90024.

Although cells in the inner plexiform layer of vertebrate retina have been shown to contain a large variety of transmitter substances, there is little evidence to indicate what these transmitters do. In order to explore this question in detail, we have begun making patch-clamp recordings from ganglion cells in cultures of adult goldfish retina. Using methods we have described (Fain et al. Vision Res. 23, 1239, 1983), we have isolated ganglion cells and maintained them in a HEPES-based minimal medium (high-K$^+$ Ringer with 10% Medium 199) at 11$^o$C. We injected the dye fast blue into the optic nerve prior to dissociation in order to identify ganglion cells. Control experiments with dye-injected intact retinas indicated that only ganglion cells were marked by this procedure. Cells identified as ganglion cells in this way had spherical or ellipsoidal cell bodies with many fine processes. The nuclei of these cells were clearly visible and contained enlarged, dark nucleoli under phase contrast. Cells were viable for several weeks though recordings were usually made within 10 days of isolation. Patch-clamp recordings were made conventionally with a List EPC 7. On-cell patches contained voltage-activated channel activity at both positive and negative pipette voltages. In whole-cell recordings, trains of action potentials could be elicited with positive current, and responses to voltage steps revealed voltage-dependent inward and outward currents which we have not as yet characterized in detail.

We have begun our investigation of the effects of putative transmitter substances on these cells with acetylcholine (Ach), since considerable evidence indicates that a subset of retinal amacrine cells contains Ach and makes direct contacts onto ganglion cells. In loose-clamp recordings, we have observed that pressure injection of 10 $\mu$M Ach onto cells in culture produces bursts of action potentials which rapidly desensitize. We investigated this response in ganglion cells recorded under voltage clamp in the whole-cell mode. The internal (pipette) solution contained 100 mM KCl or CsCl, 10 mM NaCl, 2 mM $MgSO_4$, $10^{-8}$ M free Ca, and 10 mM HEPES, pH 7.0. The external solution was normal Goldfish Ringer. When cells were voltage clamped at the resting membrane potential (-71$\pm$10 mV, n=10), Ach produced an inward current which decreased as the holding potential was made more positive and reversed near zero. Similar results were obtained when KCl in the internal solution was substituted with CsCl. These observations indicate that Ach produces an increase in conductance probably to both Na and K which would normally depolarize the cell in vivo. This result is consistent with the excitatory effects of exogenous Ach on ganglion cells in intact retina (e.g. Glickman et al., Brain Res. 234, 81).

355.14 THE PRESENCE OF ENKEPHALIN, NEUROTENSIN AND SOMATOSTATIN IN GLYCINE-ACCUMULATING AMACRINE CELLS OF THE CHICKEN RETINA. Carl B. Watt*, Hai-Biao Li and Dominic M.K. Lam. Cullen Eye Institute, Baylor College of Medicine, Houston, Texas 77030.

Recent reports have shown both peptide-transmitter (Weiler and Ball, 1984; Watt, Su and Lam, 1984) and peptide-peptide (Li, Watt and Lam, 1985) colocalization in subpopulations of retinal amacrine cells. We have continued these studies by examining the extent to which transmitter substances are colocalized in amacrine cells of the chicken retina. A comparative analysis indicated that in the chicken retina, the somas of certain enkephalin-, neurotensin- and somatostatin-immunoreactive amacrine cells are similar in size, shape and location to the somas of at least a portion of the population of cells which accumulate glycine. Taken together, these similarities were indicative of the presence of each of these neuroactive peptides in glycine-accumulating amacrine cells. A direct method to examine these possible relationships was to visualize putative peptidergic- and glycine-amacrine cells in the same retinal sections. Immunohistochemistry was used to identify enkephalin-, neurotensin- and somatostatin-cells, whereas glycine-cells were localized by high affinity $^3$H-glycine uptake-autoradiography. Such double-label studies revealed that although some amacrine cells were labelled only for glycine-uptake, others were labelled for both glycine-uptake and either enkephalin-, neurotensin- or somatostatin-immunostaining. These results, therefore, provide evidence for the presence of three subpopulations of glycine-amacrine cells which can be categorized by the coexistence of either enkephalin, neurotensin or somatostatin with glycine in these cells. Based on the earlier observation that enkephalin and neurotensin are colocalized in some amacrine cells of the chicken retina (Li et al., 1985), studies are presently underway to determine if more than one of these neuroactive peptides (enkephalin, neurotensin or somatostatin) are present in the same glycine-accumulating amacrine cell.

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355.15 ENKEPHALINS IN THE GOLDFISH RETINA: ANATOMICAL LOCALIZATION AND BIOCHEMICAL STUDIES. K.R. Fry, Y.Y.T. Su, C.B. Watt* and D.M.K. Lam. Cullen Eye Institute, Baylor College of Medicine, Houston, Texas 77030.

Studies using electrophysiological techniques have demonstrated that exogenously applied enkephalin or enekphalin analogues influence the activity of goldfish ganglion cells (Djamgoz et al., Nature 292:628, 1981). Immunohistochemical studies, however, have demonstrated only sparsely distributed enkephalin-like immunoreactive (Enk-LI) processes and, unfortunately, have been unable to identify their origin. We report here on the anatomical localization, synthesis and calcium-dependent release of enkephalin in the goldfish retina.

For immunohistochemical studies, light-adapted goldfish (Carassius auratus) retinas were fixed overnight in 4% paraformaldehyde and 0.1% glutaraldehyde in 0.1 M phosphate buffer. Vibratome sections (50-75μm) were prepared and immunostained with an antibody prepared against Leu$^5$-enkephalin (Batch A206, generously supplied by Dr. K.-J. Chang). The binding sites were visualized using the ABC-peroxidase method. Enk-LI was localized to a discrete population of amacrine cells located along the inner border of the inner nuclear layer. Processes extended into the inner plexiform layer and ramified predominantly in sublaminas 1, 3 and 5.

To study enkephalin synthesis, isolated fish retinas were incubated with $^{35}$S-methionine, homogenized in acetic acid, boiled and centrifuged at 40,000xg for 45 min. Synthesis of labelled enkephalin in the tissue was demonstrated by radioimmunoassay, thin layer chromatography and high pressure liquid chromatography. The opioid nature of the synthesized compound was confirmed in binding studies with mouse brain membrane preparations.

Enkephalin release was studied by incubating the above labelled tissue in high $K^+$ (60mM) Ringer's solution followed by repeated washings in normal Ringer's solution. The solutions were then concentrated and assayed. Approximately 8-10% of the total labelled enkephalin which had been synthesized in the tissue was found to be released. This release could be completely suppressed by 5mM $Co^{+2}$ suggesting a calcium dependent nature.

The present findings of anatomical localization, synthesis and release of enkephalin when combined with previous reports of physiological activity suggest strongly that enkephalin may be a neurotransmitter candidate for certain amacrine cells in the goldfish retina.

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355.16 IDENTIFICATION OF SUBSTANCE P IMMUNOREACTIVE GANGLION CELLS IN THE RABBIT RETINA. N. Brecha, M. Cilluffo and J. Bolz*. Dept of Medicine, Jules Stein Eye Institute, Brain Research Institute and Center for Ulcer Research and Education, UCLA School of Medicine, LA, CA 90024 and VA Medical Center, Wadsworth, LA, CA 90073.

Several neuropeptides including substance P (SP) have been localized to the mammalian retina. The majority of peptide containing retinal cells are amacrine cells. In addition, peptide containing somata in the ganglion cell layer (GCL) suggest the existence of peptidergic ganglion cells and/or displaced amacrine cells.

Normal and intraocular colchicine-treated rabbit retinas were fixed in paraformaldehyde. Sections cut perpendicular to the vitreal surface and whole retinas were incubated in a rat monoclonal antibody directed to SP and processed for either immunofluorescence or peroxidase-antiperoxidase immunohistochemistry. In several rabbits, the superior colliculus and pretectal area were injected with Fast Blue (FB) or rhodamine (TRITC) impregnated microspheres. Following a 2-5 day survival period the eye contralateral to the injection sites was treated with colchicine and subsquently fixed and processed for SP immunohistochemistry.

As we reported earlier, SP immunoreactivity in normal and colchicine-treated retinal sections is present in processes distributed to laminae 1, 3 and 5 of the inner plexiform layer (IPL). SP positive somata are present in the inner nuclear layer (INL) at the IPL border and give rise to processes to the IPL. Some of these somata also give rise to a single fine process that crosses the INL to ramify in the outer plexiform layer. A greater number of SP positive somata are present in the GCL and they also give rise to processes to the IPL. Scattered axon-like processes are observed in the optic nerve layer and optic nerve head. In retinal whole mounts this same general distribution of somata and processes is seen in all retinal regions. The highest density of immunoreactive somata is in the region of the visual streak. Well-labeled somata, which are likely to be ganglion cell bodies were observed in the GCL following injection of either FB or TRITC microspheres into midbrain visual centers. In those regions of the GCL having a dense and continuous labeling of retrogradely filled somata, some labeled somata also contained SP immunoreactivity. SP postive somata which are not retrogradely filled are also observed.

These studies confirm the presence of SP immunoreactive amacrine, displaced amacrine and interplexiform cells and directly demonstrate the presence of SP-containing ganglion cells in the rabbit retina.

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## CHEMICAL SENSORY SYSTEMS II

356.1 PROBOSCIS EXTENSION IN THE BLOWFLY: POSITIONAL EFFECTS OF CHEMORECEPTORS. S. Yetman* and G.S. Pollack (SPON: J. Marsden). Dept. of Biol., McGill Univ., Montreal, Quebec, Canada H3A 1B1.

The fly posesses several hundred chemosensilla on its labellum which are important in controlling its feeding behavior. Among these are a population of 22 identified sensilla trichodea (11 on each labellar lobe) called the "largest hairs", which are arranged along an anterior to posterior axis with largest hair #1 most anterior and #11 most posterior. We wanted to determine whether the fly made use of the positional information these hairs may be providing. Stimulation of a single largest hair with sucrose elicits proboscis extension. We tested the role of positional information by measuring the relationship between the identity of the stimulated hair and the direction of the resultant proboscis extension. Stimulation of anterior hairs elicited an anterior and slightly lateral extension of the proboscis, whereas stimulation of more lateral or posterior hairs resulted, respectively, in more laterally or posteriorly directed extension. Quantitative analysis revealed that the direction of proboscis extension is continuously graded according to the position of the hair stimulated.

To determine whether the positional information provided by the hairs had an anatomical correlate in the central nervous system, the neurons innervating each largest hair were stained by positioning a micropipette filled with cobaltous lysine on the terminal pore of the sensillum. The axons of the neurons were found to course through the labial nerve and terminate in the suboesophageal ganglion. Three distinct types of central projections were observed, each associated with a different group of hairs. The axons from hairs 1 and 2 branch in the ipsilateral hemiganglion and also project contralaterally at three distinct dorso-ventral levels. The neurons innervating hairs 3-8 terminate primarily in the ipsilateral hemiganglion where they form a 'Y' shaped pattern when viewed in the frontal plane, and have a single, dorsal contralateral projection. Finally, the neurons innervating hairs 9-11 terminate within a restricted region of the ipsilateral hemiganglion and send two projections contralaterally.

In conclusion, our behavioral observations show that the fly makes use of the positional information provided by the largest hairs. However, the directionality of the behavior cannot be explained solely by the observed projection patterns since neurons from hairs within a single morphological group have branching patterns which are indistinguishable, but nevertheless elicit differently directed extensions.

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356.2 ADAPTATION OF PRIMARY CHEMORECEPTOR CELLS: PARALLEL SHIFT OF DOSE-RESPONSE FUNCTIONS AND REDUCED VARIANCE. P.F. Borroni* and J. Atema, Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543

Chemoreceptors must extract normally signals in a noisy environment of varying background intensity. Adaptation to background conditions is a common feature of receptor cells but poorly studied in chemoreception. We used the narrowly tuned $NH_4$ cells of lobster (Homarus americanus) legs to measure the effects of self-adaptation on dose response functions over the natural range of stimulus and background concentrations. $NH_4$-sensitive cells compose the second most common narrowly tuned receptor cell population in the lobster walking legs (Johnson et al. 1984, J. Comp. Physiol. 155A:593-604). These taste organs are essential for lobster feeding behavior, and among all the compounds for which narrowly tuned receptors are known to exist, $NH_4$ is the only one that elicits feeding motions in a leg-specific behavioral bioassay (Borroni et al. submitted). During feeding the $NH_4$ receptors may encounter peak $NH_4$ concentrations as high as 20 mM in the prey tissue itself, against backgrounds of $NH_4$ varying from uM to mM.

Single cells were identified by stimulation with 5uM $NH_4Cl$ in artificial seawater containing a background of 1.6 uM $NH_4$. Extracellular chemosensory responses were recorded and counted as the number of spikes in the first 10 seconds. Dose response functions were obtained from an ascending $NH_4$ concentration series (.03uM to 30mM), in backgrounds of 1.6, 4.4, 38 and 350uM $NH_4$. Single cell dose response functions varied greatly in slope and shape and ranged over the entire 4-6 log steps tested without saturating; the variance of the response mean increased with stimulus concentration. Elevating the $NH_4$ concentration in the background had two major effects: 1) it caused a horizontal parallel shift of the dose response functions, i.e. the response to all stimulus concentrations tested was nearly equally affected; 2) it reduced single cell response intensities, suppressing mostly the cells with the strongest response. The resulting reduction in the variance of the receptor population mean response may be the main function of adaptation in these cells, which show no obvious saturation but increased variance at high stimulus intensities. In this way adaptation could extend the useful operating range of the receptors.

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356.3 ORGANIC CALCIUM ANTAGONISTS AND CERTAIN INORGANIC CATIONS INHIBIT OLFACTORY TRANSDUCTION. B.D. Winegar, E.R. Rosick, and R. Schafer. Department of Biological Sciences, North Texas State University, Denton, TX 76203.

The role of $Ca^{2+}$ in olfactory transduction was explored by aerosol application of inorganic cations and organic calcium antagonists onto the olfactory epithelium of the frog during extracellular recording. Inorganic cations that block inward calcium currents in other tissues inhibit electroolfactogram (EOG) responses. The rank order of potency of the chloride salts is $La^{3+}>Zn^{2+}>Cd^{2+}>Ca^{2+}>Al^{3+}>Sr^{2+}>Co^{2+}$. Application of $10^{-9}$ mol of $La^{3+}$ in a 2 sec aerosol mist virtually eradicates EOG responses, with only partial recovery occurring over a period of hours. $Co^{2+}$ has the least effect, producing slight and rapidly reversible inhibition at the same dosage. $Mg^{2+}$ has no effect. Brief enhancement of EOG amplitudes was observed upon application of $Ba^{2+}$, which gives greater currents than $Ca^{2+}$ in other tissues. Unexpectedly, $Ca^{2+}$ itself was inhibitory.

The organic calcium antagonists verapamil and diltiazem (2 x $10^{-10}$ mol applied over 2 sec) inhibit by approximately 70% and 50%, respectively, at 100 sec following application. The effects of both agents are reversible, with complete recovery occurring within 90 min. Another organic calcium antagonist, nitrendipine, has no action at this dosage level. Recovery from verapamil and diltiazem inhibition appears to follow a linear pattern, as does recovery from many of the inorganic cations.

The calmodulin antagonists N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W-7), trifluoperazine (TFP), and chlorpromazine (CPZ) depress EOG amplitudes with no apparent correlation to their anti-calmodulin potency. With the same dosage level as above, W-7 (the most potent calmodulin antagonist tested) has only a slight and reversible depressive action while TFP and CPZ produce apparently permanent EOG inhibition. At 100 sec after application, CPZ produces approximately 85% inhibition and TFP depresses EOGs by about 55%. Recovery from these agents approximates a hyperbolic curve, leveling off at about 25% below the pretreatment baseline for CPZ and about 45% below for TFP.

These data support the hypothesis that $Ca^{2+}$ is a current carrier and/or a second messenger in olfactory transduction. The inorganic cations inhibit EOGs in a way consistent with their blocking effectiveness on calcium channels in other tissues. The EOG enhancement observed with $Ba^{2+}$, as well as the inhibitory action of verapamil and diltiazem also support the idea that calcium channels mediate one or more components of the mechanism of olfactory transduction. An explanation for the inhibitory action of CPZ and TFP is that their amphipathic properties might enable them to disrupt hydrophobic regions of protein and lipid in the cell membrane, thus limiting the ability of receptor neurons to normally respond to odorous stimuli.

356.4 IMMUNOHISTOCHEMICAL LOCALIZATION OF AN ODORANT BINDING PROTEIN FROM BOVINE NASAL EPITHELIUM. P.B. Sklar*, J. Pevsner* and S.H. Snyder (SPON: A.L. Gundlach). The Johns Hopkins University School of Medicine, Department of Neuroscience, Baltimore, MD 21205.

Using the procedure described by Pevsner et al. (Proc. Natl. Acad. Sci. USA 82:3050-3054, 1985), we have purified a protein from bovine nasal epithelium that binds the bell pepper odorant, 2-isobutyl-3-methoxypyrazine. Rabbits were immunized with the purified protein and the resulting antiserum, when tested by immunoblotting, recognized a 19 kD polypeptide along with a minor component at 65 kD. The 65 kD contaminant cross-reacted with antiserum against bovine serum albumin. Anti-BSA antiserum, however, did not cross-react with the 19 kD band, which previously has been shown to account for the binding of [$^3$H]2-isobutyl-3-methoxypyrazine.

Immunohistochemical localization using an indirect peroxidase-antiperoxidase method in cryostat sections of bovine nasal mucosa reveals heavy staining of the glands in the lamina propria. Control experiments with normal rabbit serum and bovine serum albumin antiserum confirmed that this observed immunohistochemical pattern is specific for the odorant binding protein. The precise physiological function of this odorant binding protein still remains unclear. Its localization to the nasal glands suggests a possible role in mediating odor induced secretion. (Supported by a grant from International Flavors and Fragrances, Incorporated.)

356.5 COMPARATIVE STUDY OF ELECTROPHYSIOLOGICAL PROPERTIES OF DEVELOPMENTALLY SYNCHRONIZED POPULATIONS OF YOUNG AND MATURE OLFACTORY NEURONS IN ADULT FROG. M.S. Lidow, S.J. Kleene*, and R.C. Gesteland. Northwestern University, Evanston, IL 60201.

Because cells in the normal olfactory epithelium of adult animals are continuously turning over, it always contains not only mature receptor cells (which have established synapses with olfactory bulb cells), but also a rather large population of young receptor cells (which have not yet established such synapses). In this work we have conducted a comparative study of the electrophysiological properties of young and mature receptor cells in order to understand their contributions to the electrophysiological properties of normal frog olfactory epithelium. For this purpose we used olfactory epithelia with developmentally synchronized populations of receptor cells. To obtain such epithelia, we first ablate the epithelia by perfusing the frog nasal cavity with 0.15 M zinc sulfate ($ZnSO_4$) for 3 min. Then we allow the epithelia to regenerate for 10 days. After this, we continuously flow 50 mM hydroxyurea in Ringer (0.40 ml/day) into the nasal cavity. Thus the epithelia contain only those receptor cells which originated during the short period of time prior to hydroxyurea treatment. Since regeneration of receptor cells starts on the sixth day after $ZnSO_4$ treatment, the ages of most of these cells fall within a 4-day range. Two groups of 5 olfactory epithelia were used in the study. One group was subjected to the hydroxyurea treatment for 8 days, and thus contained only young olfactory receptor cells 8-12 days old, the axons of which presumably have not yet reached the olfactory bulb. Another group was subjected to hydroxyurea treatment for 24 days and contained a population of mature olfactory receptor cells 24-28 days old which should have synapses in olfactory bulb. The electroolfactograms (EOGs) recorded from both groups of olfactory epithelia showed no differences in polarity or shape. However, the amplitudes of EOGs recorded from epithelia with mature olfactory receptor cells were generally higher than those from young cells. We attribute this to the fact that mature frog receptor cells have much longer cilia and as a consequence larger receptor area. Extracellular recording of single cell activity showed that olfactory receptor cells of adult frogs do not pass through a stage during which they unselectively respond to all odorants. Such a stage in development has been described for embryonic rat olfactory receptor cells. When compared to more mature olfactory receptor cells, young cells had much lower (sometimes zero) spontaneous activity. Odorous stimulation of young cells evoked on the average fewer spikes, and they were much less tuned to a specific range of concentration of odorants.

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356.6 MITRAL-GRANULE CELL INTERACTIONS UNDERLIE RAT OLFACTORY BULBAR EEG. F.H. Eeckman* and W.J. Freeman (SPON: M.C. Diamond). Dept. of Physiol.-Anatomy, U.C. Berkeley, Berkeley, CA 94720.

We performed simultaneous recordings of unit pulses and EEG at various locations in the olfactory bulb of anesthetized rats. No electrical or olfactory stimulation was used. The EEG was recorded on the surface of the exposed OB with a 250 μ electrode. Using stereotaxic coordinates, a tungsten micro-electrode was positioned in the mitral cell layer directly underneath the surface electrode. (Interelectrode distance was no greater than .8 mm, which corresponds to the depth of the mitral cell layer.) The EEG was digitized at 1 msec. intervals, bandpass filtered at .3 cps to .3 kcps and stored with the pulse train. The latter was analyzed with a threshold comparator, set to emit a standard pulse 5 V, 1 msec for every signal above twice the noise level. The traces often showed 3 to 4 distinct action potential waveforms. We recorded multi-unit as well as single unit traces. Data were stored on tape and processed off line on a digital computer (Perkin Elmer 3220 mini). Power spectra were computed using the FFT algorithm. They showed 3 dominant frequency peaks in the EEG. One corresponds to the respiratory wave at 1.5 Hz. This component was filtered out using a digital filter. The second component was at 20 Hz, the third at 50-58 Hz. We used the 2 dimensional conditional pulse probability table (Freeman 1975) to find correlations between unit pulses and EEG wave amplitudes.

In all locations tested, units were found that fired with a quarter-cycle phase lead relationship to the ongoing 50 Hz frequency band of the EEG. It was noted, however, that units could be most reliably recorded from a 3 mm segment on the intermediolateral surface of the OB. Pulses correspond to mitral cell firings. The EEG trace is generated by the granule cell responses; all other cells in the bulb generate closed fields. The mitral and granule cell groups are reciprocally interconnected with excitatory-inhibitory synapses (Rall & Shepherd, 1968). A model was proposed by Freeman to explain EEG bursts on the basis of this connectivity (Freeman, 1975). Our data showing a fixed time relationship with quarter-cycle time lead for pulses to waves provide further evidence for this model, as well as extending this evidence to another species.

Rall, W. & Shepherd, G.W. *J.Neurophysiology 31*:884-915, 1968.
Freeman, W.J. *Mass Action in the Nervous System* (Academic Press), 1975.

356.7 TIME-SERIES ANALYSIS OF RABBIT OLFACTORY BULB EEG: BURSTS AND INTERBURSTS. K.A.Grajski and W.J.Freeman. Group in Biophysics. Dept. of Physiology. UC Berkeley. Berkeley, CA 94720.

The olfactory bulbar EEG of waking rabbits alternates between bursting and interbursting states. The alternation occurs at the respiratory rate (2-8Hz) with bursts occurring with inhalation. As an initial assay in the search for odor-specific neural activity manifest by the EEG of rabbits trained with odor CSs, we evaluated the differential temporal, spectral and spatial characters of these states in naive rabbits.

The bulbar field potentials were recorded epidurally from the lateral aspect of the olfactory bulb of chronically implanted rabbits (N=7) with an 8 by 8 electrode array. From successive 6 second trials, burst and interburst segments (100ms) were selected. For statistical analysis, the sample of bursts and interbursts each form a class. The correlation (Fisher z-transformed) between the mean burst and interburst spatial patterns of RMS amplitude averaged 1.35 across animals. Pairwise correlations of spatial patterns within classes averaged 0.92 +/- 0.4 and 0.7 +/- 0.4 for bursts and interbursts, respectively. These results suggest that the spatial distribution of EEG in the bulb is common to both states, and in each segment there is a global waveform.

The averaged time series power spectrum for bursts and interbursts had characteristic peaks. For burst segments, maximal power occurred at 50-70Hz, a half-maximal peak near 20-40Hz and rapid decline near 90Hz. For interburst segments, broad peaks of equal height occurred near 50-70Hz and 20-40Hz, but were never greater than burst segment peaks. Spread of peaks, as measured by one-half maximal peak power, averaged 30Hz for bursts and 80Hz for interbursts. In short, the burst spectra are focused near a recurring peak frequency, while interbursts show a broad power spectrum.

Recent work suggests that odor-specific information is restricted to particular frequency domains of the EEG. It is manifested by statistically stable temporal, spectral and spatial-spectral properties across burst segments recorded weeks apart. The generation of EEG is modelled by a set of nonlinear differential equations which display equilibrium, limit cycle and chaotic states. Bursts correspond to limit cycle activity, interbursts to chaos. The alternation of burst and interburst activity is interpreted as the repeated emergence and collapse of an ordered state from a chaotic state driven by the periodic surge of receptor activity with inhalation.

356.8 GLOMERULAR TERMINATIONS OF OLFACTORY RECEPTOR AXONS IN A SNAIL TENTACLE. Barbara Tolloczko* and R. Chase. Department of Biology, McGill University, Montreal, Quebec, H3A 1B1, Canada.

One of the most striking examples of evolutionary convergence in neuroanatomy is the synaptic glomeruli which constitute the terminal sites for olfactory afferent fibers in both vertebrates and crustacea. In order to determine whether the generality of this observation extends also to the molluscs, we have investigated the course of receptor axons in the tentacular olfactory system of the terrestrial snail, Achatina fulica. The receptor neurons were selectively labelled by application of HRP crystals to the surface of the olfactory epithelium or by iontophoretic injection of HRP into the sub-epithelial lobules containing receptor cell somata. The tentacles were examined in serial sections with both LM and EM.

The receptor axons terminate at three different locations: 1) the tentacle ganglion, 2) the digit-like peripheral extensions of the tentacle ganglion, and 3) the procerebral lobe of the brain. We have observed glomeruli only in the digits. Here, when seen in transverse sections, they consistently occupy a medial and ventral position. The fasciculated axon bundles stream down from the lobules above, turn nearly 90° to head towards the tentacle ganglion, and then terminate in one of the glomeruli. The glomeruli are recognized by their glial wrapping, the absence of cell bodies, and the prevalence of synaptic contacts. The ultrastructure of the synapses is similar to that of central chemical synapses in other gastropod ganglia, with symmetrical membrane thickenings and asymmetrical accumulations of predominately small (50 um) lucent vesicles. The cell bodies of interneurons sometimes occupy periglomerular positions. The glomeruli measure about 25 µm by 50 µm in cross section, and extend 60-190 µm along the length of the digit. There are about 6 glomeruli in each of the 5 digits which underlie the sensory epithelium.

The morphological features of the glomeruli in the snail tentacle are similar to those present in the vertebrate olfactory bulb and in the insect deutocerebrum . It therefore appears that there is a compartmentalization of afferent inputs in all well-developed olfactory systems. The generality of this structural plan suggests a relationship to the requirements of information processing in olfaction.

356.9 ROLE OF OLFACTORY AND VOMERONASAL SENSES ON GARTER SNAKE RESPONSE TO AIRBORNE ODORANTS. J.Halpern*, E. Erichsen* and M. Halpern. Department of Anatomy and Cell Biology, Downstate Medical Center, Brooklyn, N.Y. 11203.

Twenty garter snakes were tested for responsiveness to airborne odorants in an olfactometer (designed and constructed by B. Slotnick). Dry, deodorized air was passed through saturation tubes containing concentrated amyl acetate, limonene, earthworm wash, fish water, distilled water or through tubes containing a live earthworm or a live fish. This odorized air, mixed with a constant stream of air, passed directly into a 1 liter glass chamber in which the snake was tested. A test session consisted of a 5 minute adaptation period followed by 2 trials separated by a 3 minute intertrial interval. A trial consisted of 3 one minute intervals in which tongue flicks were counted prior to, during and following a one minute odor or control ($dH_2O$) stimulus. In any given session an odor was always tested with a control stimulus. The order of testing was systmatically varied. All snakes were tested once with each odor prior to surgery and once with each odor following surgery. Preoperatively, snakes significantly increased tongue flick rates(TFR) during presentation of all six odor stimuli. TFR increases were greatest during limonene and amyl acetate and least during earthworm wash presentations. After preoperative testing the snakes were tested for locomotor activity in an open field apparatus and then subjected to either bilateral vomeronasal (N=7), bilateral olfactory (N=7) nerve cuts or sham (N=6) surgery. Following surgery snakes were again tested in an open field apparatus. There was a slight postoperative decrease in locomotor activity for all groups following surgery with no differential lesion effects. Snakes with sham lesions retained differential responding to odor stimuli following surgery. Both vomeronasal and olfactory nerve groups showed a significant decrement in TFR to odor stimuli postoperatively, however, the olfactory nerve group did not significantly increase TFR in the presence of odor whereas the vomeronasal nerve group did to a slight, but significant, extent.

These results suggest that the increased TFR in response to airborne odorants observed previously (Burghardt, 1977;1979; Halpern & Kubie, 1983 ) may be mediated by both olfactory and vomeronasal systems in snakes. Since olfactory nerve lesions appear to have a more devastating effect on the response to airborne odorants, it is possible that the initial increase in TFR following odor stimulation depends on it, whereas the maintained increase in TFR depends on a functional vomeronasal system. This is the first demonstration of a deficit following olfactory nerve lesions in snakes and is consistent with the idea that the olfactory system is involved in the detection of volatile odorants.

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356.10 OLFACTORY STIMULI AND THE EFFECTS ON GLUCOSE, INSULIN AND GROWTH HORMONE IN CHICKENS. D.H. Krestel-Rickert, and C.A. Baile. Washington University School of Medicine, Dept. of Preventive Medicine, St. Louis, MO 63167.

Olfaction has been proposed as a means of determining growth rate and affecting hormones in some animals. In this study several classes of olfactory stimuli (Ketones, esters, alcohols, etc.) were investigated for their potential to alter hormonal (eg. growth hormone) and metabolite (eg. glucose) concentrations in chickens. In addition, insulin concentrations were also considered during the fed and fasted states. Blood samples were drawn from broiler chicks (4-9 weeks) at -4 hr (after access to food and 4 hr prior to odor), 0 min (time of odor exposure and end of 4 hr fast) and post-meal/odor states of +15 and +30 minutes. At time 0, birds were allowed access to food as an olfactory stimulus was delivered to the nares of the chicken at a flow rate of approximately 4 ml/min. Chicken plasma was assayed using a chicken standard (Litron Labs, 50 IU/mg) in a heterologous RIA. Five replications for 10 birds/day were made for each odorant tested. Growth hormone levels for all odors tested except butyric acid (an aliphatic acid) were not significantly altered. Mean values of GH for butyric acid were significantly decreased from time 0 (odor/meal exposure) to time 15 (15 min post odor/meal: 72.65 ng/ml vs 31.95 ng/ml, $p<.05$). No significant differences existed for either glucose or insulin concentrations for butyric acid. This decrease may be attributed to butyric acid since similar changes in GH concentration were not associated with other odorants tested or the fed/fasted state. The possibility exists that some olfactory stimuli may alter growth hormone concentrations in poultry and in turn effect the normal growth pattern over time; on the other hand, feed odorants could provide a means of stimulating pituitary hormones to effect both behaviors and metabolism.

356.11 BEHAVIORAL CONSEQUENCES OF THE AMILORIDE-SUPPRESSED SALT TASTE RESPONSE. B. K. Formaker*, K. S. White* and D. L. Hill, (SPON: E. DuBrul), Dept. of Psychology, Univ. of Toledo, OH 43606

Neurophysiological recordings from the rat chorda tympani nerve reveal that lingual application of amiloride suppresses gustatory responses to NaCl. The effects of amiloride are proposed to be specific to sodium transduction processes. Although the magnitude of response suppression to NaCl is as much as 80%, complete suppression has not been achieved. This indicates that additional transduction processes must occur. To learn if the residual NaCl response after amiloride is qualitatively similar to the unsuppressed NaCl response, a conditioned taste aversion procedure was used.

Adult rats were maintained on a 23.5 hr. water deprivation schedule throughout the experiment. The number of licks/10s were measured to the following randomly presented 10 stimuli: 0.1M and 0.5M NaCl, $NH_4Cl$ and KCl; 0.1M Na-saccharin, 0.1M citric acid, 1.0M sucrose and 0.01N HCl. This measurement was taken one day before and one day after aversion conditioning. Avoidance training in rats preexposed to amiloride was conducted by first exposing the animal to ten, 10s trials of 100 μM amiloride hydrochloride. Preexposure to amiloride was immediately followed by alternating 10s amiloride trials with 10s of either 0.1M NaCl, 0.5M NaCl or 0.1M $NH_4Cl$ for a total of 20 CS presentations. Immediately after the last CS presentation, the animal was injected with 0.3M LiCl (1% B.W.' i p.). Conditioning without amiloride preexposure consisted of alternating the same stimuli described above with distilled water for a total of 30 CS presentations. Six rats were used in each group. Suppression ratios were measured by dividing the mean number of post-test licks by the mean number of pre-test licks. Generalization curves for each CS was obtained by comparing the suppression ratio for that stimulus to the suppression ratios for each of the remaining nine stimuli.

Rats trained to avoid 0.1M $NH_4CL$ or 0.5M NaCl after amiloride learned aversions similar to rats that were not preexposed to amiloride. In contrast, rats trained to avoid 0.1M NaCl after amiloride were similar to saline-injected controls. Unexpectedly, rats conditioned to avoid 0.5M NaCl after amiloride suppressed to all salts with the exception of 0.1M NaCl. This is in contrast to rats conditioned to 0.5M NaCl alone, which only suppressed to 0.1M and 0.5M NaCl. These results indicate that the residual response to 0.1M NaCl after amiloride is not sufficient to condition a taste aversion and that the residual response to 0.5M NaCl is qualitatively different than 0.5M NaCl alone.

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356.12 TIME COURSE OF RESPONSES TO SUCROSE AND NACL ELICITED FROM STIMULATING THE ANTERIOR TONGUE AND NASOINCISOR DUCTS. S.P. Travers and R. Norgren. Dept. of Behavioral Science, College of Medicine, Pennsylvania State University, Hershey, PA 17033.

Previous work from this laboratory has established that, for gustatory neurons in the nucleus of the solitary tract (NST) of the rat, the optimal sapid chemical may vary depending upon which subpopulation of oral taste receptors is stimulated. Specifically, of the four basic taste stimuli, sucrose is most effective for nasoincisor duct, and NaCl is most effective for anterior tongue stimulation. These initial observations were based on the magnitude of these responses, defined as the number of impulses/5s. The present analysis demonstrates that the time course of these responses also differs. We analyzed responses of 26 single neurons in the NST to 1.0M sucrose and 0.3M NaCl applied to the anterior tongue and nasoincisor ducts. Impulses were accumulated in post-stimulus time histograms (PSTH, 500ms bins) for 10s following stimulus onset. PSTHs were averaged across animals to obtain mean time courses. When the anterior tongue was stimulated with sucrose, the response peaked slowly, in the 13th bin (6.0-6.5s) following response onset and a greater number of spikes occurred during the second, as compared with the first, 5s of the response. In contrast, when sucrose was applied to the nasoincisor ducts, the activity of NST neurons peaked in the third bin (1.0-1.5s) following response onset and more impulses occurred in the first 5s of the response. Sodium chloride applied to the anterior tongue also elicited a relatively rapid response that peaked in the second bin (0.5-1.0s) following response onset. Similarly, the response to NaCl applied to the nasoincisor ducts peaked in the second bin, but the magnitude of this response was much smaller than any of the previously discussed responses. These time course differences were confirmed using correlation coefficients (Pearson's r). The time course of the nasoincisor duct response to sucrose was negatively correlated with the response to sucrose applied to the anterior tongue ($r=-.39$, n.s.) but positively correlated with the response to NaCl applied to the anterior tongue ($r=+.78$, $p<.05$). Behavioral studies have demonstrated that rats can discriminate taste quality within 200-700ms following stimulation (Halpern and Tapper, 1971; Scott, 1974), which suggests that the early portion of the neural taste response is sufficient for coding taste quality. The robust phasic response to stimulation of the nasoincisor ducts with sucrose is consistent with the hypothesis that these receptors are important for coding the sweet quality, particularly for initial detection. The location of nasoincisor duct receptors in the anterior oral cavity would further enhance their effectiveness in the rapid discrimination of sweet-tasting substances. (Supported by NIH NS07121 and NS20397.)

356.13 POLYSACCHARIDE TASTE AND APPETITE IN THE RAT. A. Sclafani, H. Hertwig*, J.W. Nissenbaum* and M. Vigorito (SPON: I. Abramov). Dept. of Psychology, Brooklyn College and Graduate School of City University of New York, Brooklyn, NY 11210.

Rats display an avid appetite for starch-derived polysaccharides (eg., Polycose). In fact, at low molar concentrations rats are more attracted to Polycose solutions than they are to sucrose solutions as indicated by two-bottle preference tests. This appetite is due to the oral rather than the postingestive effects of Polycose since rats consume large amounts of a Polycose solution even when it drains out an open gastric fistula. That it is the taste rather than the smell of Polycose that rats find attractive is indicated by the findings that experimentally- produced aguesia suppresses Polycose intake much more than does experimentally-produced anosmia. Conditioned taste aversion experiments revealed that rats taste Polycose as qualitatively different from sucrose.

Polycose contains saccharides (glucose polymers) of varying chain lengths and it is not clear which saccharides rats find the most "tasty." This question was addressed by measuring the taste preferences of adult female rats during brief (3-min) two-bottle preference tests. The rats were given the choice of solutions (.0125 molar) containing one of the following saccharides: glucose (a monosaccharide), maltose (a dissaccharide), maltotriose (a trisaccharide), maltooligosaccharide (a mixture of saccharides 4 to 8 glucose units in length), and Polycose (a mixture containing saccharides of 1 to 30+ glucose units in length).

The results indicated that the rat's order of preference for the shorter saccharides was as follows: maltooligosaccharide > maltotriose = maltose > glucose. The rats displayed a nonsignificant preference for maltooligosaccharide over Polycose. These findings demonstrate that saccharides of four or more glucose units in length maximally stimulate the rat's polysaccharide appetite, while shorter saccharides are less effective.

In another experiment nondeprived rats were given a 30-min two-jar choice test using powdered Polycose vs. corn starch, the latter contains only large polysaccharide molecules. The rats significantly preferred the corn starch to the Polycose. This finding was unexpected in view of the solution results and whether it is the taste or some other property of starch (texture, smell) that rats find attractive remains to be established.

Taken together, these behavioral findings indicate that rats, in addition to their well known taste for sugars, also have a taste and appetite for starch-derived polysaccharides.

356.14 THE EFFECTS OF STIMULUS SELECTION AND CLASSIFICATION CRITERIA ON THE EXISTENCE OF GUSTATORY NEURON TYPES IN THE RAT NTS. G. P. Mark* and T. R. Scott. Dept. of Psychology and Inst. for Neuroscience, Univ. of Delaware, Newark, DE, 19716.

We studied the impact of two factors on conclusions regarding the existence of gustatory neuron types in the rat nucleus tractus solitarius (NTS): (1) The stimulus array used to generate neuronal response profiles, and (2) The criterion for defining a related cluster of such profiles. Records were taken from 42 NTS cells responding to an array of 16 naturally occuring compounds. Profiles for each cell were determined according to (a) it's responses to this full array, or (b) to a condensed set composed of the four prototypical stimuli (NaCl, sucrose, HCl, quinine HCl). In each condition, profiles among all neurons were intercorrelated and multidimensional scaling techniques and hierarchial cluster analyses were used to study similarities among them. The tendency of response profiles to fall into groups was assessed by asking two statistical questions: (a) Are the profiles randomly distributed across the taste domain? or the reverse, (b) Is each profile identical to all others within a local cluster? When profiles were based only on responses to the four prototypical stimuli, the answer to the first question was decidedly negative. The probability that neuronal profiles were randomly distributed was $p<.01$. The answer to the second question was rather positive. Thirty-eight of the 42 neurons could not be excluded from membership in one of only four clusters of profiles. This represents support for the existence of gustatory neuron types. However, when profiles were based on responses to all 16 stimuli, clustering was less evident. The first question was still answered in the negative: neuronal response profiles are not randomly distributed ($p<.05$). However, the answer to the second became more equivocal: only 12 of 42 neurons were not excluded from four clusters when the membership criterion was that profiles be identical to within the limits of experimental error. It is not clear why an enlarged stimulus array regularly causes clusters to degenerate, as has been shown in several experiments. More stimuli should simply define response profiles more accurately, with no prejudice toward whether this should increase or decrease similarities among them. Perhaps if complex taste stimuli are composed of elements (sweet, salt, sour, bitter?) these are combined differently by individual cells such that these qualities would permit greater discrimination among response profiles. If this tendency persists, the existence of neuron types in a functioning system responding to hundreds of taste qualities becomes doubtful. The criterion for clustering is also crucial to answering the question of whether neuron types exist. It is clear that response profiles are not randomly distributed across tastes. However, it is difficult to demonstrate that they fall into a small number of groups (neuron types) within which they are identical.

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356.15 STIMULATION OF SHAM FEEDING IN THE RAT BY SUCROSE, MALTOSE, GLUCOSE, AND FRUCTOSE. K. Joyner*, G.P. Smith, R. Shindledecker* and C. Pfaffmann. Dept. of Psychiatry, Cornell Univ. Medical College and Eating Disorders Institute and E.W. Bourne Behavioral Research Laboratory, New York Hospital, White Plains, NY 10605 and Rockefeller University, New York, NY 10021.

Sham feeding provides normal orosensory stimulation by sugars, but minimizes their postingestive negative feedback effects. Sham feeding of sucrose has been demonstrated to be a biphasic (Mook, 1963;), monotonic (Weingarten & Watson, 1982) or sigmoid function (Bernz et al, 1983) of concentration. We compared the potency of sucrose, maltose, glucose, and fructose to stimulate sham feeding in chronic gastric fistula rats (n=7) after 17 h of food deprivation and after no food deprivation. The following concentrations of all sugars were presented in ascending order: 0.1, 0.2, 0.4 and 0.8M. After 17 h of food deprivation, the rank order of potency for the stimulation of sham intake (ml-30 $min^{-1}$) was sucrose (strongest), maltose, glucose and fructose (weakest). There was one exception to this order: 0.1M maltose elicited a larger intake than 0.1M sucrose.

After no deprivation, there was a similar rank order of potency, but the curves relating sham intake to the individual sugars were shifted to the right by approximately one log concentration. In the non-deprived condition, 0.1M and 0.2M maltose elicited intakes that were equivalent to those elicited by 0.1M and 0.2M sucrose.

When the individual curves were analyzed for peak rate of change of intake per log change of sugar concentration, deprivation increased the effect of glucose ($p<.05$), but not that of the other sugars: The ratio of deprived to non-deprived peak rate of change of intake was 1.8 for glucose, 1.2 for sucrose, 1.0 for maltose, and 0.8 for fructose.

The results demonstrate (1) that deprivation increases the sensitivity of the sham feeding response to these four sugars, and increases the peak rate of change intake of glucose; and (2) that the maltose paradox (Pfaffmann, 1982) is present during sham feeding at 0.1M and 0.2M in deprived and non-deprived conditions.

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356.16 CAPSAICIN SUPPRESSES DEPILATORY INDUCED IRRITATION. T.R. LAHANN* (SPON: A. SNOW). School of Pharmacy, Washington State University, Pullman, WA 99164-6510.

Capsaicin is now recognized as a relatively selective sensory neurotoxin. Injected s.c. into rodents, it produces major alterations in the neurochemistry, morphology and function of primary afferent neurons. Capsaicin also blocks some (but not all) types of cutaneous inflammation. The literature suggests that this anti-inflammatory activity may be a functional consequence of capsaicin's action on sensory neurons.

The studies reported here demonstrate that capsaicin blocks depilatory induced irritation in rats. A mild irritant response is observed after a single topical application of thioglycolate based depilatory preparations. Severe irritation develops following two or more depilations over a 4-day period. The causative factor of this irritant response appears to be the thioglycolate.

Thioglycolate (depilatory) irritation was quantitated 4 hours after the last depilation by subjectively evaluating the extent of scale and eschar formation. A 5 point grading scale was used. The observed irritation was not blocked by acetylcholine or histamine antagonists. Also, neither a topically applied nonsteroidal anti-inflammatory drug (indomethacin) nor a topical steroid (flucinolone acetonide) blocked development of the irritation. However, topical capsaicin applied in an ethanol: Tween 80:saline (48:4:48) vehicle suppressed development of the irritant response. The vehicle alone had no suppressive effect. Capsaicin's anti-irritant effect was evident when it was applied either before or after the first depilation. It was most effective, however, when applied shortly after the initial depilation. This anti-irritant action was dependent upon the amount of capsaicin applied per application, the number of capsaicin applications, and the time at which it was first applied. For example, when a 2% capsaicin solution was applied over a 6 hour period immediately following the initial depilation, the development of the irritation reaction was reduced by more than 90%.

Capsaicin's anti-irritant action does not appear to be restricted to the immediate site of its application. Even if applied to only one-quarter of the depilated area, capsaicin still effectively suppressed irritation over the entire depilated region. This expanded action does not appear to result from oral ingestion (Elizabethan collars prevented oral ingestion) or from smearing of the topical preparation, yet parenteral administration of capsaicin was only partially effective in antagonizing the depilatory induced irritation.

These data suggest that depilatory irritation is not dependent upon "classical" inflammatory mediators, but may be mediated by the peripheral nervous system. They also suggest a novel approach to the control of depilatory-induced irritation.

## LIMBIC SYSTEM AND HYPOTHALAMUS I

357.1 BASIC ORGANIZATION AND CHOLINERGIC FEATURES OF THE FELINE ISLANDS OF CALLEJA COMPLEX (ICC). K. Talbot*(1), N.J. Woolf (1) and L.L. Butcher (1,2). Psychology Dept. (1) and Brain Res. Inst.(2), UCLA, Los Angeles, CA 90024.

The granular islands of Calleja in the olfactory tubercle are accompanied by more diffusely arranged satellite neurons. This ensemble of granular and non-granular elements - the ICC of Fallon (Brain Res. Bull. 10:775, 1983) - has been described only in the rat. We report here, however, that the complex is differently and more discretely organized in the cat, where it is also more uniformly filled with an exceptionally dense cholinergic neuropil.

Unlike the case in rats, nearly all the Callejal islands in cats lie near or partially within the tubercular molecular layer. In coronal Nissl material (N = 6), these were seen to undergo progressive changes laterομedially, allowing differentiation of at least four types of islands: lateral, intermediate, medial, and tubercular-accumbal. They differ in their degree of exposure to the tubercular molecular layer, horizontal flattening, and granule cell density and cavitation. They also differ in the type and density of their satellite cells. The aggregation of these medium to large cells in hilar spaces above (and/or enclosed by) each Callejal island completes the division of the feline ICC into discrete units not commonly observed in rats or macaque monkeys.

Many satellite (but no granule) cells were found to be lightly or moderately reactive for choline acetyltransferase (ChAT) in monoclonal immunofluorescence preparations (N = 5) and for acetylcholinesterase (AChE) in pharmaco-histochemic material (N = 4). These cells were counted in 40 μm coronal sections from the rostral to the caudal pole of the olfactory tubercle, yielding 1,243 ChAT cells in 7 sections and 2,545 AChE cells in 14 sections. In both cases, over 80% of the cells were confined to hilar ICC areas (as opposed to intra-, peri-, and sub-granular ICC sectors). The vast majority were polymorphic neurons 14-28 μm in length, distinctly less voluminous than striatal ChAT or AChE cells. Their mean number rose in statistically significant ($p<.05$) increments laterομedially (e.g., from lateral to intermediate to tubercular-accumbal ICC units, the mean number of ChAT neurons increased from 4.9 to 12.8 to 29.2).

A concomitant increase in the cholinergic neuropil from lateral to medial ICC units suggested that the ChAT and AChE cells concentrated in their hilar areas are local circuit neurons. This view was reinforced, though not proved, by the observation of many descending enzyme processes from hilar areas toward a previously unreported band of intense ChAT and AChE neuropil just below most granular islands. These unusual subpial bands are virtually absent in olfactory tubercle areas between Callejal islands and appear to represent the densest cholinergic terminal fields in the forebrain.

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357.2 ENTORHINAL TARGET NEURONS OF OLFACTORY BULB EFFERENT FIBERS: ANTEROGRADE DEGENERATION, GOLGI IMPREGNATION, GAD-IMMUNOCYTOCHEMISTRY AND ELECTRON MICROSCOPY COMBINED IN RAT.

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Fibers originating in the olfactory bulb terminate superficially in layer I of the entorhinal area (EA). To identify target neurons of this projection the anterograde degeneration technique was combined with Golgi-electron microscopy of presumed projection neurons (Golgi-EM) and with immunoelectron microscopy of presumed, GABA-ergic interneurons (incubation with an antiserum against glutamic acid decarboxylase; GAD-EM). Lesion of the olfactory bulb results in electron-dense degeneration of axons and axon terminals. In early stages of degeneration the terminals remain synaptically attached to their target neurons. Two to three days following surgical removal of the olfactory bulb, young adult rats were perfused transcardially with formaldehyde/glutaraldehyde mixtures. For Golgi-EM, the dissected brains were impregnated according to Golgi-rapid and Golgi-Kopsch procedures. Twenty-five well-Golgi-impregnated EA-neurons of various classes were embedded in Epon, serially thin sectioned and screened in the EM for the presence of degenerating synaptic contacts. For GAD-EM, 40 um thick Vibratome sections were incubated with antiserum against glutamic acid decarboxylase according to an indirect procedure (Sternberger, 1979; Oertel et al, 1981). After embedding in Epon, 25 immunoreactive neurons in layer Ia of EA were serially thin sectioned and screened for the presence of degenerating axo-somatic synaptic contacts.

In the Golgi-EM material, degenerating terminals were observed forming asymmetric synaptic junctions with distal dendrites of layer II sparsely spinous pyramidal cells located in the dorsolateral subdivision (DLEA) of EA, and with distal dendrites of layers II and III spinous pyramidal cells located in the ventrolateral subdivision (VLEA) of EA. On nearly all screened GAD-immunoreactive cells, both in DLEA and in VLEA, we observed degenerating terminals, also with asymmetric synaptic junctions. These neurons are presumably small multipolar, local circuit neurons. Immunoreactive boutons form symmetric synapses in layer I both with immunoreactive neurons and with non-immunoreactive dendritic profiles. It is likely, therefore, that the GAD-immunoreactive, presumably GABA-ergic layer I neurons which receive olfactory bulb input take part in microcircuits providing feed-forward inhibition to pyramidal cells receiving olfactory bulb input.

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357.3 INTERHEMISPHERIC CONNECTIONS OF THE HIPPOCAMPAL FORMATION IN THE BAT, ANTROZOUS PALLIDUS. L.J. Beasley*, G.O. Ivy, M. Leon and G. Lynch. Dept. of Psychobiology and CNLM, Univ. of Calif., Irvine, CA 92717

There have been few studies to date on the origin of hippocampal commissural projections in mammals and none in bats. Since bats are known to have a well developed hippocampus and to be adept at spatial memory tasks, it is of interest to examine one of the major projection systems of this brain region.

Four pallid bats were anesthetized and the skull overlying the hippocampus was removed unilaterally. Multiple small injections of HRP were placed along the septo-temporal extent of hippocampus. The bats were perfused the following day with a mixed aldehyde fixative. The brains were then removed and alternate sections were processed with TMB and BDHC histochemistry.

While the sizes and exact placements of the injections varied somewhat among the 4 bats, the combined results indicate that cells in every subregion along most of the septo-temporal axis of hippocampus project to the opposite hemisphere. Thus, labeled cells were found in all subregions of CA1, CA2, CA3 and CA4 as well as in prosubiculum and subiculum. No labeled granule cells were seen in the dentate gyrus. In approximately the anterior third of the hippocampus apparently all of the neurons in CA1 through CA4 were labeled. However, in more posterior regions the relative density of labeled neurons decreased in CA1 such that by approximately the middle third of hippocampus only 1/2 to 1/3 of these cells were labeled; these were located proximal to CA2. Still more posteriorly, CA1 neurons were only labeled in heavily injected cases. It appears that the CA1 projection is rather sparse, since neurons in this area were not labeled in one bat which received a smaller injection of HRP. In extreme posterior portions of hippocampus the cells of CA4 were densely labeled, while subicular neurons were lightly labeled despite dense HRP injections contralaterally.

In two of the bats there was distinct anterograde transport of HRP to the commissural zone. A fine granular reaction product covered the inner 37% of stratum moleculare of dentate gyrus and the inner 53% of this zone in CA1. Comparable figures for HRP transport in the rat are 38% and 70%, respectively (Ivy, pers. obs).

In conclusion, as is the case in other mammals,the commissural projection of hippocampus arises principally from regions CA3 and CA4 and terminates on the proximal portion of pyramidal and granule cell dendrites. However, unlike the case in the rat, all fields of Ammon's horn project to the contralateral hemisphere in the pallid bat. Further, the commissural terminal zone occupies a smaller proportion of the CA1 dendritic field in the bat than the rat. Supported by NIH NS21484 (to M.L.) and NICHD-HD06551 (L.J.B.).

357.4 A HRP STUDY OF BRAINSTEM AFFERENTS TO THE MEDIAL SEPTUM-DIAGONAL BAND NUCLEUS OF THE RAT. Robert P. Vertes. Mercer University School of Medicine, Macon, GA 31207

Cells of the medial septum-vertical limb of the diagonal band nucleus (MS-DB) have been shown to discharge synchronously in phase with hippocampal theta to pace this rhythm. Disruption of the rhythmical discharge of MS-DB neurons results in hippocampal desynchronization. It has been shown that MS-DB activity is, in turn, controlled by nuclei of the brainstem (Vertes, Prog. Neurobiol. 19: 159, 1982). In an attempt to further localize the sites of origin and trace the ascending trajectory of brainstem neurons involved in modulating the septum-hippocampus, we placed HRP injections in the MS-DB and analyzed retrograde transport to cells of the brainstem. Small WGA-HRP injections were made in 25 rats and brains were reacted using the TMB procedure of de Olmos et al. (J. Comp. Neurol. 181:23, 1978). Brainstem nuclei labeled following MS-DB injections included (from caudal to rostral): A1 noradrenergic area; cells directly adjacent to the MLF at the level of the rostral pole of the inferior olive; nucleus gigantocellularis, pars alpha (dorsal to the pyramids and ventral to NGC proper); retrofacial nucleus-C1 adrenergic area (ventrolateral medullary tegmentum directly caudal to the facial nucleus); raphe magnus; nucleus incertus (on the midline in the medullary gray at the level of the locus coeruleus); dorsolateral tegmental nucleus of Castaldi (DLT) (co-extensive and medial to LC in the pontomedullary gray); locus coeruleus, nucleus subcoeruleus; raphe pontis; medial aspect of pontis oralis; lateral parabrachial nucleus; Kolliker-Fuse nucleus; dorsal raphe; median raphe (MR); scattered cells in midbrain dopaminergic fields (retrorubral, VTA and caudal linear nuclei); prominent cell group in the mesencephalic gray directly lateral to the aqueduct; nucleus of Darkschewitsch and the supramammillary nucleus (SUM). In the hypothalamus, labeled cells were observed in the posterior, dorsal and lateral groups and in the caudal magnocellular and submammillothalmic (lateral to the mammillothalmic tract) nuclei. The most densely labeled areas were: raphe nuclei (magnus, pontis and median groups); incertus, DLT, LC, nucleus of Darkschewitsch, SUM and each of the hypothalamic nuclei.

We previously reported (J. Neurophysiol. 46:1140, 1981) that the most effective brainstem sites for producing hippocampal synchronization (theta) and desynchronization were pontis oralis and the median raphe, respectively. The present results of strong MR labeling suggests a direct influence of the median raphe on the MS-DB in the modulation of hippocampal slow-wave activity. The relatively light RPO labeling, on the other hand, suggests di- or multi-synaptic pathways from pontis oralis to MS-DB possibly through the supramammillary nucleus which was very heavily labeled in the present report.

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357.5 NEOCORTICAL AFFERENTS TO THE LIMBIC CORTEX OF THE CAT. M. Pérez-Bas*, C. Cavada* and F. Reinoso-Suárez. Dept. Morfología, Fac. Medicina, Univ. Autónoma de Madrid, 28029 Madrid, Spain.

The limbic cortex (LC) is classically considered a relay station in the Papez circuit, and as such a link between the anterior thalamic nuclei and the hippocampal formation. Recently, LC has been shown to be a major source of projections to neocortical association areas, notably to the prefrontal cortex (PFC). However, little is known about the precise origin of the neural input to LC other than that arising from the thalamus. In the present study, the neocortical areas that send associational connections to LC were mapped using the retrograde transport of HRP. Single or multiple injections of the enzyme were made in each of the five subdivisions of the cat's LC, i.e., in the infralimbic (Il), prelimbic (Pl), anterior limbic (LA), cingulate (Cg) and retrosplenial (Rs) areas.

Following injections in Il and the ventral portion of Pl, labeled neurons were observed in medial and lateroventral PFC, insular cortex (IC, mainly in the agranular subdivisions), caudal half of the sylvian sulcus (SS), perirhinal cortex (PrhC), in LA, and in the portion of area 6 situated in the medial surface of the hemisphere. Injections placed in dorsal Pl labeled neurons in many of these same regions, including the PFC (with the labeling more dorsally situated than in the preceding cases), IC (mainly in the granular subdivision), SS, PrhC, LA, and medial area 6. However, dorsal Pl injections also labeled neurons in Il, cortex bordering the anterior ectosylvian sulcus (SEsA), Cg, and a few scattered cells in parieto-temporo-occipital association areas. Similar to injections in Il and Pl, injections in LA labeled neurons in PFC (especially dorsally), and in areas 6, Il, Pl and Cg. Further, low numbers of HRP-positive neurons were observed in the splenial visual area (VS), Rs, IC, SEsA, area 7, lateral suprasylvian area (LS), suprasylvian fringe (SsF), posterior ectosylvian area (Ep), posterior suprasylvian area (Ps), area 20 and PrhC. Following injections in Cg, neuronal labeling was observed in PFC, medial area 6, Pl, AL, Rs, VS, IC, SEsA, SsF, LS, areas 7, 19, 20, Ep and Ps, and in PrhC. Some of the areas containing labeled neurons and the densities of labeling varied, however, depending upon the portion of Cg injected. Following injections in Rs, labeled cells were observed in LA, Cg, ventral PFC, areas 7 and 20, and in PrhC.

These findings indicate that the largest and most consistent source of neocortical input to LC arises from the PFC and the subdivisions of LC itself. The premotor cortex, and monomodal and polymodal association cortices provide additional afferents to LC, with the Cg area being the major recipient of the connections from the association cortices. Although the various limbic areas have several common sources of neocortical afferents, the overall pattern of these connections is specific for each area.

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357.6 COMPARISON OF HISTOCHEMICAL AND CYTOCHEMICAL COMPARTMENTS IN THE AMYGDALOID COMPLEX: A HUMAN AND ANIMAL STUDY. K.B. Sims*, R.S. Williams. Neuropathology, Shriver Center, Waltham, MA. 02154

The amygdala is a multimodal relay for fibers from the frontal and temporal association cortex, basal forebrain, diencephalon, striatum and brainstem. Abnormalities in the structure and function of the amygdala are known to occur in Alzheimer disease and temporal lobe epilepsy, and may be present in schizophrenia and autism. Neuropathological study of the human amygdala is hampered by the absence of a comprehensive and consensus definition of its nuclear subdivisions, and uncertainty regarding homology with anatomic subdivisions and interconnections experimentally defined in laboratory animals.

Serially adjacent sections of the human amygdala were stained with Nissl or histochemical methods for acetylcholinesterase (ACE), nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d) and cytochrome oxidase (CO). ACE and NADPH-d stained the neuropil differentially. The borders of these histochemical compartments coincided with those of the principal amygdaloid nuclei in Nissl stains. For example, the basolateral nucleus stained intensely with ACE and poorly with NADPH-d, and the central nucleus stained poorly with both methods. The relatively sharp borders of the ACE and NADPH-d compartments also facilitated confirmation of more subtle nuclear boundaries and the identification of additional nuclear subdivisions in Nissl stains. The CO method, by contrast, stained central and basal nuclei intensely, but differential staining of other nuclear boundaries was not sharp.

The NADPH-d method stained selected neurons intensely in Golgi-like fashion, and the density of stained cells varied in different nuclear subdivisions. For example, few to no NADPH-d positive cells were found in central and basal nuclei while cell density was much greater in the adjacnt lateral, medial and accesory basal nuclei. In the striatum NADPH-d positive cells are labelled with antibodies to somatostatin and neuropeptide Y (Kowall et al, unpublished), but similar data is not yet available for the amygdala.

The histochemical staining patterns in the human amygdala were compared to those of the mouse, ferret and Rhesus monkey. Differential neuropil and cell staining was the same in the principal nuclei of all species.

In summary, ACE and NADPH-d histochemical stains enhance the definition of Nissl cytoarchitectural boundaries in the amygdaloid complex, delineate nuclear subdivisions within the human amygdala and facilitate comparison of homologous subdivisions between mammallian species. They may therefore facilitate the neuropathological examination of the amygdala in human neuropsychiatric diseases. Supported by NIMH 31154.

357.7 CONNECTIONS OF THE AMYGDALA WITH THE HIPPOCAMPUS AND ENTORHINAL CORTEX IN THE MONKEY. R.C. Saunders and D.L. Rosene, Dept of Anatomy, Boston Univ Sch Med, Boston, MA, 02118.

The hippocampal formation (HF) has long been considered important in memory function and more recently, the amygdala (AMYG) has also been implicated. To the extent that both the AMYG and the HF contribute to memory function, then their interconnections either direct or via the adjacent entorhinal cortex (EC) may be of considerable functional importance. While much is known about the important afferents to the HF from the EC, much less is known of the interconnections of the AMYG with the HF and EC.

Using fluorescent retrograde tracers and tritiated amino acids, the interconnections of the AMYG, HF and EC were investigated in the rhesus monkey. In the HF, the subicular region is the major recipient of afferents originating in the lateral basal, accessory basal and medial basal nuclei of the amygdaloid complex. In turn the subiculum of the HF sends a direct projection to the medial basal and accessory basal nuclei of the AMYG. The EC receives projections from the lateral, lateral basal, accessory basal and the medial basal nuclei of the AMYG as well as from the subiculum, presubiculum and CA1 region of the HF. The adjacent prorhinal cortex receives afferents from only the lateral and lateral basal nuclei. The perirhinal cortex receives afferents from only the lateral basal and the accessory basal nuclei. The projection to the prorhinal cortex and the perirhinal cortex from the HF originates from the superficial layers prosubiculum and CA1 region. Within the EC the afferent termination from the HF and AMYG are segregated in distinct laminae. The HF fibers terminate in layers 5 and 6, while afferents from the AMYG terminate in layers 2 and 3. Prorhinal cortex receives afferent fibers from both the AMYG and the HF with overlapping termination in layers 1, 2 and 3 and additional termination in layer 5 from the subiculum. The perirhinal cortex receives afferents from the HF, which terminate in layers 2, 3 and 5 as well as from the amygdala which terminate in layer 1. In addition to the EC projections to the dentate gyrus and CA fields the perirhinal cortex (layers 4 and 5) and the prorhinal cortex (layers 2, 3 and 4) send efferents directly to the subiculum and prosubiculum, i.e., the same region as the amygdaloid projection to the HF.

It is of considerable functional interest that the major areas within the amygdala and the hippocampus which are interconnected, (the lateral basal, medial basal and accessory basal nuclei of the AMYG and the prosubiculum and subiculum of the HF) either directly or via the surrounding entorhinal cortical areas (including the prorhinal and perirhinal cortex), are also important as the sites of origin of efferent projections to subcortical nuclei which have been implicated in memory function (e.g. mammillary bodies, nucleus medialis dorsalis of the thalamus and the nucleus basalis).

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357.8 VASOACTIVE INTESTINAL POLYPEPTIDE PROJECTIONS TO CENTRAL NUCLEUS OF AMYGDALA FROM SUPRACHIASMATIC NUCLEUS

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The central nucleus of the amygdala (CNA), a limbic structure involved in autonomic integration, contains a dense plexus of vasoactive intestinal polypeptide (VIP) immunoreactive fibers, but very few immunoreactive cell bodies (Gray, et. al., Neuroscience 11:339(1984)). In our ongoing analysis of the neuropeptidergic projection fields of CNA with other autonomic centers, we have observed that the course of VIP fibers from CNA was directed medially and rostrally toward hypothalamus. It is known that within the hypothalamus, the suprachiasmatic nucleus (SCN), a critical site for circadian regulation, contains a population of VIP cells in its ventrolateral aspect.

In an effort to determine if VIP fibers in CNA originate from cells within SCN, the retrograde tracer true blue was injected unilaterally into CNA of adult male, Wistar rats. After an appropriate survival time for transport of the tracer, the animals were treated with a 1% colchicine solution, administered intracerebroventricularly, and were sacrificed 24 hours later. Perfused brains were sectioned on a vibrating microtome at 30 μm and the tissue was processed for VIP immunofluorescence.

Injections that were localized to CNA resulted in retrograde labeling of a small population of VIP containing cells within the SCN. These double-labeled cells were distributed primarily in the ventral and lateral aspects of the ipsilateral SCN. A few cells labeled with true blue alone were also observed within dorsomedial SCN.

These data demonstrate the presence of a direct VIP projection from SCN to CNA, although contributions to this CNA plexus from other VIP cell populations are also possible. This neuropeptidergic system may provide a significant link through which circadian information can access limbic-autonomic regulatory centers within CNA.

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357.9 Amygdalospinal Projections In The Cat: A Retrograde Transport Study Using Rhodamine Labeled Latex Microspheres. B.B. Sandrew*, D.L. Edwards and W.E. Foote. Departments of Neurosurgery and Psychiatry, Stanley Cobb Neuroscience Laboratory, Massachusetts General Hospital and Harvard Med. Sch., Boston, MA 01124.

There is ample evidence from retrograde transport studies in several animal species that amygdalofugal fibers arising from the central nucleus (Ce), descend the neuraxis to terminate within the mesencephalon and pontomedullary brainstem. However recent anatomical evidence based on HRP methodology has shown that the Ce of the macaque monkey contains neurons which project directly to the spinal cord (Mizuno et al, Neurosci. Lett., 53:327-330, 1985). This finding adds a new dimension to the anatomical and physiological significance of the limbic forebrain which warrants further study toward a more comprehensive understanding of amygdala function. Since a good deal of current knowledge pertaining to the amygdala has been discovered through studies of the cat we considered it especially important to confirm the presence of amygdalospinal projections in that species using a new, highly sensitive fluorescent retrograde tracer. Rhodamine labeled fluorescent latex microspheres (Beads, Katz et al, Nature, 310:498-500, 1984) were then injected below C1 into the spinal cord of 5 cats. After survival periods of 14 to 28 days animals were sacrificed coronal 40um frozen sections were cut. Spinal cord injections were found to be restricted to the needle paths with little diffusion. In all cases the injections involved dorsal and ventral quadrants of the spinal cord including the dorsal and ventral horns within C1 and C2. Bead labeled cells were concentrated within a well circumscribed area corresponding to the Ce of the amygdala. In addition, at levels in which the greatest amount of labeling was noted within the Ce distribution of fluorescent cells extended into the medial nucleus. The greatest number of Ce cells in which Beads could be detected ranged between 44 to 67/section, whereas the medial nucleus was found to have no more than 5 labeled cells within its borders in any section. In a sample of neurons (n=30) taken at various AP levels of the Ce, the dimensions of labelled soma (approximated under 25X fluorescence to be 17um +/- 5.58 s.e.m. by 29.73 um +/- 1.25 s.e.m.) indicate that amygdalospinal efferents labeled with Beads were large cells. These data show that nuclei within the cat amygdala contribute direct projections to the spinal cord. Specifically, high cervical injections resulted in retrograde labeling of a dense, well circumscribed cell group comprising the entire rostrocaudal extent of the Ce with a modest labeling in the medial nucleus. Supported by Grant NS 22549 awarded to WEF.

357.10 ORGANIZATION OF AMYGDALOID PROJECTIONS TO THE DORSOMEDIAL THALAMUS AND PREFRONTAL CORTEX IN THE RAT. A.J. McDonald. Dept. of Anatomy, Univ. of So. Car. Sch. of Med., Columbia, SC 29208.

Previous studies have shown that the amygdala projects to both the dorsomedial thalamic nucleus (DM) and its cortical projection area, the prefrontal cortex (PFC). In this investigation rats (n=28) received injections of different fluorescent retrograde tracers (true blue and diamidino yellow) into DM and either the sulcal (PFCS), polar (PFCP), or medial (PFCM) prefrontal cortex in order to examine the relationship of amygdaloid neurons with cortical and/or thalamic projections. PFC injections labeled neurons in the basolateral (BL), basomedial (BM), ventral endopiriform (Env), and rostral lateral nuclei as well as the periamygdaloid cortex (PAC) and the medial part of the amygdalo-hippocampal area (AHA). In BL, which contained the great majority of neurons projecting to PFC, most labeled cells were concentrated in particular parts of the nucleus and were topographically organized. Neurons with projections to PFCM were concentrated in dorsal and medial parts of both the anterior (BLa) and posterior (BLp) subdivisions of BL. Neurons with projections to PFCP were concentrated in the ventral part of the rostral two-thirds of BL. Neurons with projections to PFCS were concentrated in the rostral half of BLa (except its lateral edge) and the ventrolateral corner of BLp. The overwhelming majority of labeled neurons in BL were large pyramidal or piriform cells that correspond to class I neurons described in Golgi studies. Occasional small neurons with thin dendrites were also observed; these cells may be class II neurons. DM injections labeled numerous cells in the anterior division of the cortical nucleus, medial nucleus, and the adjacent caudomedial part of the central nucleus. Moderate numbers of labeled cells were found in caudal portions of BM and PAC, whereas scattered cells were observed throughout the rest of the amygdala with the exception of the lateral nucleus. In BL and AHA many DM-projecting neurons were observed along nuclear boundaries and in the adjacent white matter. Neurons in BL, BM and AHA usually had large elongated or irregular somata and 2-4 primary dendrites that branched sparingly. Other cells had smaller ovoid somata. The morphology and distribution of DM-projecting cells in the basolateral amygdala indicates that they are primarily large class II neurons. Double labeled neurons, projecting to both DM and PFC, were not observed unless the cingulate cortex dorsal to DM was inadvertently labeled by the injection needle. These findings suggest that different neuronal populations in the amygdala project to the two poles of the DM-PFC system. In BL class I neurons are the predominant cell type involved in PFC projections, whereas class II neurons, hitherto thought to be primarily local circuit neurons, project to DM. (Supported by NIH Grant NS-19733.)

357.11 BASAL FOREBRAIN EFFERENTS TO THE MEDIAL DORSAL THALAMIC NUCLEUS IN THE RHESUS MONKEY. K. K. Hreib*, D. L. Rosene and M. B. Moss. Dept. of Anatomy, Boston Univ. Sch. of Med., Boston, MA 02118.

Subcortical efferent connections of the basal forebrain (BF), [septal area (SA), diagonal band (DB), and nucleus basalis (NB)] were investigated in eight rhesus monkeys. In four animals, injections of tritiated amino acids (TAA) were placed in the BF posterior to the olfactory tubercle and below the anterior commissure. In one of the cases, the injection was centered in the anteromedial part of NB with slight involvement of the horizontal limb of the DB. In another case, the injection involved the anterolateral part of NB with no involvement of the DB. In a third case, the TAA injection was more posterior in NB beneath the anterior commissure. In all three cases extensive anterograde termination was observed in the ventral half of the magnocellular part of the medial dorsal thalamic nucleus (MD) with the heaviest termination posteriorly. In the fourth case, the TAA injection involved the ventral part of the SA as well as the vertical limb of the DB and showed extensive termination that extended more dorsally in magnocellular MD (MDmc) than the other three cases. To determine which of the diverse neuronal types in the BF give rise to these thalamic projections, in three monkeys injections of horseradish peroxidase (HRP) were placed into MD. In two cases, the injection was centered in MDmc with minimal involvement of the parvocellular part (MDpc). In a third case, the injection involved MDpc with minimal involvement of MDmc. In the cases with injections in the MDmc, labeled neurons were observed throughout the full extent of NB, DB and SA with the greatest number of cells in the DB. On the other hand, in the case with the HRP injection in MDpc, only a few scattered cells were labeled in the BF. In order to determine if the BF cells projecting to MD, were like the cortically projecting cells, acetylcholinesterase (AChE) positive, one of the MDmc cases was prepared for the simultaneous visualization of HRP and AChE. We observed that roughly 30% of the neurons in NB and DB that were HRP positive were also AChE positive, but in the SA a smaller proportion were double labeled. This double labeling was observed with a new procedure that utilized the TMB stabilization method (Rye, J Histochem Cytochem, 1984). While AChE is not an absolute marker for cholinergic neurons, studies have shown that in the BF almost 90% of the cells that are AChE positive are also positive for choline acetyltransferase (Eckenstein, J Neurosci, 1983). Hence, it is likely that the basal forebrain projection to MD is cholinergic. In order to determine if the NB projection to MD is a collateral of NB projections to cortex, a monkey was injected with fluorescent retrograde tracers in orbitofrontal cortex and MD. This case showed that approximately 50% of the BF neurons that project to orbitofrontal cortex also project to MDmc.

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357.12 DIFFERENTIAL PROJECTIONS FROM CINGULATE AND RETROSPLENIAL SUBDIVISIONS OF THE POSTERIOR CINGULATE GYRUS TO THE MES- AND DIENCEPHALON OF THE RABBIT. J.L. Bassett and T.W. Berger Psychobiology Program, Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260

Efferent projections from the posterior cingulate gyrus to the mes- and diencephalon were studied using autoradiography. Cytoarchitectonic subdivisions of the cingulate gyrus were defined according to Rose and Woolsey (J. Comp. Neurol., 89, 1949). The present study deals with the granular (posterior) cingulate and retrosplenial subdivisions.

A series of small injections of 3H-proline (0.3ul, 75uCi/ul) into either the cingulate or retrosplenial divisions of the gyrus were made along its rostrocaudal extent. Following a 30-40 hr survival period, animals were sacrificed and their brains processed according to Cowan et al. (Brain Res., 37, 1972). Analysis of autoradiographic material revealed several differences in relative projection strengths and patterns of termination.

Terminal labeling was most dense in the anteromedial (AM) and anteroventral (AV) thalamic nuclei. Within AM, labeling was greatest after injections localized in the anterior portions of the posterior gyrus, whereas silver grain densities in AV were greatest after injections in the mid to posterior gyrus. Silver grains in lesser density also were seen in the anterodorsal nucleus and were greatest after injections in posterior cingulate and mid to posterior retrosplenial. Moderate to dense silver grain deposits were seen in the ventrolateral (VL), lateroposterior (LP) and laterodorsal (LD) nuclei. Labeling in VL was greatest after cingulate injections and was topographically organized such that rostrally placed injections resulted in the greatest densities of labeling rostrally, and more caudally located injections with densities greatest caudally. Although labeled terminals were arranged similarly within LP and LD, each cingulate subdivision contributed equally to this projection. A similar pattern of silver grains also was detected in the mediodorsal and mediodventral nuclei but was less dense. Silver grain deposits were also seen in the zona incerta and filiform nucleus. Injections in the retrosplenial subdivision yielded a greater density and longer rostrocaudal extent of silver grains in these regions than did cingulate injections.

Terminal labeling in the mesencephalon included: the pretectal area (PTA), deep layers of the superior colliculus (SP) and the central gray (CG). Although the location of silver grain deposits in the PTA and SP did not vary with differing injection locations, labeling in CG was seen only after retrosplenial injections. Label was also seen in lateral and central tegmental areas after rostral cingulate and retrosplenial injections and in the posterior thalamus after injections in the posterior portions of these subdivisions. Supported by NSF(83BNS-16464).

357.13 ADENOSINE DEAMINASE-CONTAINING PROJECTIONS FROM THE POSTERIOR HYPOTHALAMUS TO TRIGEMINAL MESENCEPHALIC PRIMARY AFFERENT NEURONS IN THE RAT. J.I. Nagy[1] and P.E. Daddona[2]* Dept. of Physiology, Univ. of Manitoba, Winnipeg, Man., Canada, R3E 0W3[1] and Dept. of Internal Medicine, Univ. of Michigan Med. Sch., Ann Arbor, 48109 MI.[2]

The mesencephalic nucleus of the trigeminal nerve (Mes V) harbors large primary afferent neurons which convey proprioceptive and mechanoreceptive input from jaw closing muscles and periodontal ligaments, respectively. It has been known for a long time that, unlike their counterparts in dorsal root ganglia, the somas of these neurons receive chemical synaptic contacts. However, the source of these synaptic terminals has not been determined. In our immunohistochemical analysis of the distribution of adenosine deaminase (ADA) in rat brain we found that, although mesencephalic neurons were themselves devoid of immunostaining for this enzyme, their surface was covered with a rich plexus of ADA-immunoreactive fiber-like structures. Occasionally, ADA-immunoreactive axons were seen travelling towards and making contact with mesencephalic neurons. The source of the ADA-containing elements surrounding neurons in Mes V was investigated using indirect immunofluorescence and peroxidase-antiperoxidase staining techniques in combination with retrograde fluorescence tracing with fast blue (FB) or lesion methods.

ADA-immunofluorescence staining was observed around mesencephalic neurons which were simultaneously retrogradely labelled with FB after injection of this dye into the masseter muscles of mastication. Injection of FB into Mes V resulted in the FB labelling of ADA-immunoreactive neurons in the posterior basal hypothalamus. Ablation of the posterior hypothalamus by radiofrequency lesions abolished ADA-immunostaining around mesencephalic neurons.

These results indicate that ADA-containing neurons in the posterior hypothalamus innervate mesencephalic primary sensory neurons which project, in part, to muscles of mastication. It is suggested that the hypothalamus, via a direct action on these sensory neurons, may exert autonomic control over jaw movements. Moreover, given their large size and accessibility, it appears that mesencephalic neurons may provide an ideal model system for electrophysiological investigations of the neurotransmitter(s) utilized by ADA-containing hypothalamic projections which are now known to include diverse areas of the brain (Nagy et al., Science 224, 166-168, 1984).

357.14 THE ANTERIOR THALAMIC NUCLEI IN ANTHROPOID PRIMATES: A GOLGI STUDY. E. Armstrong. Anatomy Department, Louisana State Univ. Med.Ctr., New Orleans, La.70112

Previous studies have shown that the human anterior principal complex (AP: anteroventral and anteromedial nuclei) contains many more neurons than expected for a nonhuman anthropoid whose brain is scaled to human dimensions. To determine whether the increase in numbers of neurons also involved changes in the size or configuration of individual neurons, the dentritic trees of AP neurons were analyzed following a Golgi impregnation of human and nonhuman anthropoid brains.

All material came from adult specimens. The perfused brains (Saimiri, number of specimens (N) =3, Aotus N=3 and Macaca N=3) were stained following the Golgi-Braittenberg method. The adult human (N=3) and chimpanzee (N=1) brains which had been routinely fixed in 10% neutral buffered formalin were impregnated with a new Golgi variant (Armstrong and Parker, in preparation). Aotus brains stained with both methods were compared and found to have no significant differences. Both methods were used and a Sholl analysis was used to quantitatively compare the dendritic sizes among the specimens.

Large multipolar neurons were observed in all the anthropoids and are the focus of this report. The dendritic trees of these anthropoids did not differ in their overall shape or appearance. No statistically significant difference in the number of primary branches was determined. The human AP neurons, however, have many more branches than those in the monkey brains and the human-monkey difference is largest at 60-80 um from the perikaryal center.

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357.15 THE LATERAL HYPOTHALAMIC PROJECTION TO DORSAL RAPHE IS EXCITATORY. M.R. Park, Department of Anatomy, University of Tennessee Center for the Health Sciences, Memphis, TN 38163.

The dorsal raphe nucleus receives forebrain afferents from just two sources, the lateral habenula and the hypothalamus, principally the lateral hypothalamic area. Intracellular recording experiments were undertaken in the rat to examine the input from lateral hypothalamic area, which until now has not been studied using physiological methods. Male Long-Evans rats were anesthetized with urethane (induction with a single dose of 1.0 g/kg, i.p.) and ketamine (40 g/kg., i.m., given hourly). Glass recording pipettes, filled with 4% horseradish peroxidase, for intracellular labeling, in 0.5 M Tris buffer, pH 7.6, and 0.5 M potassium methyl sulphate, were positioned in the dorsal raphe nucleus. Bipolar stimulus electrodes were placed in the lateral hypothalamic area using stereotaxic procedures.

The following observations apply to the slow firing dorsal raphe neurons with broad action potentials that are presumed to be serotonergic. Electrical stimulation of the lateral hypothalamic area, using brief (50-100 us) square wave constant current pulses 50-500uA in amplitude, produces an initial depolarizing response followed by a hyperpolarization. The depolarization is graded, can be reversed by the injection of depolarizing current, and increases in amplitude with hyperpolarization. It therefore has all the properties of an excitatory postsynaptic potential (EPSP) caused by increase in membrane ionic conductance. Similarly, the hyperpolarization is a conductance-increase inhibitory postsynaptic potential (IPSP). It is graded, increases with depolarizing current injection, and can be reversed by hyperpolarization. The IPSP most likely results from activation of the recurrent inhibitory circuit of dorsal raphe. When suprathreshold, the EPSP triggers a single action potential and after-hyperpolarization. The latter adds to the IPSP to produce a hyperpolarization that is deeper than that of the IPSP alone. The latency of the EPSP is 6-10ms. The straight line distance from stimulus to recording site, which is a good approximation of the actual axonal pathway, is approximately 4 mm, so that the fibers from lateral hypothalamus to dorsal raphe have conduction velocities in the order of 2 m/s. The EPSP latency does not change with change in stimulus strength, from which we can conclude that the response is monosynaptic.

The innervation from lateral hypothalamic area is dense and the response it evokes is correspondingly large when activated synchronously. However, even this unnatural activation of the pathway does not trigger more than a single action potential. Our data show that this is by far the major forebrain input to dorsal raphe and is the only input known to be excitatory.

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357.16 HYPOTHALAMIC PROJECTION TO THE DORSAL RAPHE NUCLEUS IN THE RAT: AN ANTEROGRADE STUDY USING THE PHAL METHOD, R.L. Cowan* and M.R. Park (SPON: T. Bertorini). Department of Anatomy, University of Tennessee Center for the Health Sciences, Memphis, TN 38163.

Experiments utilizing degeneration, anterograde autoradiographic, and retrograde labeling techniques have shown a projection to the dorsal raphe nucleus from hypothalamus. We have used a new anterograde labeling technique to study the course, density, and topography of this projection.

Iontophoretic injections of phaseolus vulgaris leucoagglutinin (PHAL) were made in the lateral hypothalamic area of male Long-Evans rats. The original protocol of Gerfen and Sawchenko (Brain Res., 290:219, 1984) for injection and immunohistochemical detection of the lectin was closely followed. Survival times ranged from 7 to 14 days.

Three forms of labeling are produced. Confined to the vicinity of the injection site is a cloud of extracellular label typical of lectins. Within this cloud scattered neuronal somata and dendrites are darkly labeled. The intracellular incorporation of PHAL by these cells most likely accounts for all of the anterograde axonal labeling seen, the third form of labeling.

Terminal labeling following injections centered in lateral hypothalamic area, with some spread to anterior hypothalamus, is primarily ipsilateral and is as follows. Fibers coursing via medial forebrain bundle produce terminals in lateral and medial preoptic area, bed nucleus of the stria terminalis, ventral pallidum, substantia innominata, anterior amygdala, accumbens, septal nuclei, thalamic reticular nucleus, and zona incerta. The lateral habenula is reached via the stria terminalis and fibers passing through the fields of Forel reach periventricular grey and the dorsal raphe. Additional terminals were observed in the nucleus of the diagonal band, supraoptic, parabrachial and dorsal parabrachial nuclei, superior colliculus, thalamus, and reticular formation.

The dorsal raphe nucleus receives fibers via two different hypothalamic efferent pathways. A dorsal fiber group exits the hypothalamus and curves caudally and dorsally, entering the dorsal raphe nucleus, primarily at its rostral tip. A second, slightly smaller fiber group travels ventrally and caudally, ascends through the medial longitudinal fasciculus, pierces it perpendicularly, and enters the ventral aspect of dorsal raphe. Terminal axons having numerous varicosities are present throughout the rostral-caudal extent of dorsal raphe. A particularly dense innervation is present immediately ventral to the ependymal cell layer of the fourth ventricle. Many fibers course parallel to the rostral-caudal axis of the brain in this region.

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357.17 LACK OF INHIBITORY EFFECT OF CYSTEAMINE (B-MERCAPTOETHYLAMINE,CSH) ON PITUITARY AND HYPOTHALAMIC VASOPRESSIN CONTENT WHEN ADMINISTERED CHRONICALLY. J. Haldar, I. A. Aral and G. Abram. Dept. of Biol. Sc, St. John's Univ., New York, NY 11439 and Dept. of Neurology, Columbia Univ., New york, NY 10032

Injection of Cysteamine hydrochloride (CSH) to rats decreases plasma and tissue somatostatin and prolactin. Recent work, based on $^{35}S$ Cysteine incorporation studies has suggested that CSH inhibits the biosynthesis and induces the degradation of oxytocin (OT) but not of vasopressin (VP). Work from our laboratory has demonstrated that. following an acute CSH injection during rehydration which was preceeded by dehydration, both OT, and VP levels of the hypothalamus decrease. To determine, whether chronic CSH administration would reduce the VP in the pituitary and hypothalamus content more effectively the following experiments were performed. All experiments were done on male Long Evans rats. Animals were divided into 5 groups. Control (I), 72 hrs. dehydration (II), 72 hrs. dehydration followed by 72 hrs. rehydration (III), 72 hrs. dehydration followed by 72 hrs. rehydration together with 90mg/kg/day sc CSH(IV), and the same dose of CSH in control rats (V). CSH was administered via osmotic minipumps. All rats were decapitated, the pituitary and hypothalami were isolated, homogenized in 0.1 N HCl and centrifuged. The supernatant fluid was used for the determination of VP by radioimmunoassay. Our results show (1) 72 hrs. dehydration caused 70% reduction of pituitary but not of hypothalami VP content. (2) Rehydration increased both pituitary and hypothalamic VP content, pituitaryto control level and hypothalamus to a level higher than control (3) chronic CSH administration together with rehydration did not decrease either pituitary or hypothalamic VP content. We, therefore, conclude that although acute CSH administration effectively reduces VP content of the hypothalamus, chronic CSH fails to do so. This lack of effect of CSH in the chronic experiment is perhaps due to a quicker degradation of CSH in the animals. ( Supported by NIH, HD-13147 and an Instrumental grant from NSF).

357.18 ESTROGEN TARGET NEURONS IN RAT HIPPOCAMPAL FORMATION. R. Loy and B. S. McEwen. Department of Anatomy, University of Rochester Medical Center, Rochester, NY 14642 and Department of Neurobiology, Rockefeller University, New York, NY 10021.

Many studies are finding sex differences in transmitter and receptor levels, in dendritic plasticity during development, in capacity for post-lesion axonal reorganization, and in other aspects of hippocampal function. To localize specific cell populations which may be responsible for this sexual dimorphism, we examined the dentate gyrus, Ammon's horn, subiculum, and entorhinal cortex autoradiographically in female rats following treatment with (3H)-estradiol.

Estrogen sensitive neurons are located in the deep layers of the entorhinal cortex, and in pyramidal cells of the ventral and dorsal subiculum, ventral CA1 and CA3. Within the dorsal CA1 estrogen target cells are most likely interneurons, in stratum oriens immediately subjacent to stratum pyramidale, and in stratum radiatum. Very few cells in dorsal CA3 accumulate (3H)-estradiol.

The major cell type labeled with (3H)-estradiol in the dentate gyrus is not the granule cell but hilar interneurons in the infragranular zone. As in Ammon's horn, many more cells in ventral regions than in dorsal regions accumulate estrogen.

While estrogen-sensitive neurons are not numerous in the hippocampal formation, these cells are concentrated within specific subpopulations of neurons, including pyramidal neurons in the subiculum and entorhinal cortex, and interneurons in CA1 and the dentate gyrus. We and others have found that the levels of norepinephrine and choline acetyltransferase, as well as adrenergic and cholinergic receptors, are sexually dimorphic or sensitive to estrogen levels in the hippocampus. Interneurons may be targets for both the cholinergic and adrenergic afferent systems, suggesting that transmitter activity may be regulated locally by estrogen-sensitive target neurons.

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357.19 SEX DIFFERENCES IN $^{125}$I-CCK-OCTAPEPTIDE BINDING SITES IN THE VENTROMEDIAL NUCLEUS. T.R. Akesson, P.W. Mantyh, C.R. Mantyh*, D. Matt*, and P.E. Micevych. Dept. of Anatomy and Medicine and the Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

Although there is a good correlation between the levels of gonadal steroids and sex differences in reproductive behavior and food intake, the neurochemical mechanisms by which gonadal steroids may regulate these behaviors remain unknown. An important central nervous system site for steroid modulation of food intake and reproduction is the ventromedial nucleus of the hypothalamus (VMN), which contains neurons that accumulate both androgens and estrogens. Recently, it has been suggested that some of the activities of the VMN are dependent on the neuroactive peptide cholecystokinin octapeptide (CCK). High levels of CCK in the VMN have been measured by radioimmunoassay, and immunohistochemical analysis has revealed a dense plexus of CCK fibers and terminals which appose and make synaptic contact with VMN neurons. A majority of the CCK fiber terminals in the VMN originate in the ipsilateral, dorsal parabrachial nucleus (PBN), a relay station in the ascending pathway of brainstem visceral and taste stimuli projecting to the VMN and rostral forebrain. The present study examines distribution of CCK binding in relation to differences in endocrine state.

Adult male and female rats as well as females on each day of the estrous cycle were sacrificed, the brains removed, and sectioned in the coronal plane. Specific high affinity $^{125}$I-CCK binding sites were then localized and quantified using the serial slide-mounted sections of VMN and PBN in conjunction with autoradiographic techniques and LKB Ultrafilm. The VMN of females contained significantly greater levels of CCK binding sites than did males of similar age whereas the levels of CCK binding sites in the cerebral cortex and striatum were similar. These data are consistent with the hypothesis that activity of CCK in the VMN is influenced by gonadal steroids. This influence could be affected by steroid hormone modulation of CCK receptors postsynaptic to cholecystokinergic imput from the PBN. These variations may provide a neurochemical basis for a number of sexually dimorphic behaviors which have been linked to the VMN.

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357.20 EFFECT OF GONADAL STEROID HORMONES ON LEVELS OF CHOLECYSTOKININ IN THE HYPOTHALAMUS. P. Micevych, V.L.W. Go* and D. Matt*, Dept. of Anatomy and the Laboratory of Neuroendocrinology, Brain Research Institute, UCLA, Los Angeles, CA 90024, and the Dept. of Gastroenterology, Mayo Clinic, Rochester, MN 55905.

Sex differences in food intake (FI) and body weight are temporally correlated with levels of gonadal steroids. Male rats are hyperphagic compared to females. Body weight and FI of females increase during diestrus, when estrogen is elevated, and decrease during proestrus, when estrogen is low compared to progesterone. Although the regulation of feeding is a complex behavior involving oropharyngeal and gastric mechanisms, various CNS loci have been implicated in this regulation, including the ventromedial nucleus (VMN), paraventricular nucleus (PVN), and the lateral hypothalamic area. The VMN and PVN appear to be neural targets for the effects of gonadal steroids on FI. Recently, the neuroactive peptide cholecystokinin (CCK) has been suggested as a central satiety factor at the level of the VMN and PVN. The purpose of the present study was to determine whether variations in gonadal steroids are correlated with tissue content of CCK in specific hypothalamic regions. Because substance P and bombesin have also been linked to the control of FI, these neuropeptides were also analyzed. Normal male (220-250g) and female (200-220g) rats were decapitated, the brains removed and 500 µm coronal sections obtained through the hypothalamus. The medial preoptic nucleus, bed nucleus of the stria terminalis, PVN, VMN, lateral hypothalamic area and median eminence were microdissected and radioimmunoassayed for CCK, substance P and bombesin. Tissue levels of CCK in the medial preoptic nucleus (39.8±4.1 pmole CCK equivalents/gm) were elevated in males compared to females (30.1±5.2 pmole CCK equivalents/gm), while the VMN contained more CCK than the male (29.3±7.6 and 20.8±1.5, respectively). Moreover, CCK levels in the female varied during the estrous cycle in the VMN, bed nucleus of the stria terminalis and median eminence. CCK levels in the VMN and median eminence were highest on diestrus day one (65.8±14.6 and 35.3±2.1 pmole CCK equivalents/gm, respectively), while the CCK content in the stria terminalis, an area containing CCK cell bodies, peaked 24 hrs earlier during estrus (50.6±15.3 pmole CCK equivalents/gm). The concentration of bombesin did not vary during the estrous cycle and substance P levels changed only in the VMN where levels co-varied with CCK, highest on diestrus day one (46.0±5.6) and lowest on estrus (16.9±4.7 pmole CCK equivalents/gm). These results indicate that gonadal steroids may regulate the levels of CCK and substance P but not bombesin in specific areas of the hypothalamus. Thus steroids, especially estrogen, may facilitate the release of the satiety factor CCK in the hypothalamus which may help to explain the hypophagia observed in rats during periods of elevated estrogen.

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357.21 BIDIRECTIONAL PATHWAYS BETWEEN THE MEDIAL PREOPTIC NUCLEUS AND OTHER SEXUALLY DIMORPHIC NUCLEI. R.B. Simerly and L.W. Swanson. The Salk Institute, La Jolla, CA 92037.

The medial preoptic nucleus is a sexually dimorphic complex composed of 3 distinct subdivisions (Simerly et al., '84). Although the full functional significance of this nucleus remains obscure there is general agreement that it plays a critical role in the neural control of male reproductive behavior, and perhaps participates in other sexually differentiated functions as well. While examining the neural circuitry associated with the MPN we have found that it shares bidirectional connections with several nuclei that are known to posses structural sexual dimorphisms.

Injections of the retrograde tracers true blue, SITS, or WGA into the MPN resulted in retrogradely labeled cells in many brain regions. Those containing the highest densities of labeled cells included the encapsulated part of the bed nucleus of the stria terminalis (BSTe), the medial amygdaloid nucleus (MeA), the ventrolateral septum (LSv), the anteroventral periventricular nucleus (AVPv), the ventral part of the ventromedial nucleus (VMHv), and the arcuate nucleus (ARH). Projections from each of these nuclei to the MPN were confirmed autoradiographically, or by using PHA-L as an anterogradely transported axonal tracer (Gerfen and Sawchenko, '84). Further, the projections from these regions are distributed topographically within the MPN in accordance with the cytoarchitectonically defined subdivisions.

In each region the distribution of retrogradely labeled cells showed a remarkable correspondence to the distribution of steroid concentrating cells (Pfaff and Keiner, '73; Stumpf, '79). In addition, each of these nuclei are known to posses structural sexual dimorphisms. The MPN, the MeA, BSTe and AVPv are sexually dimorphic with respect to volume (Gorski, '84 for review), and steroid sensitive sexual dimorphisms exist in the pattern of specific inputs to the LSv, ARH, MeA and AVPv; while in the VMH, sex differences in nucleolar and nuclear size of individual neurons have been found. (DeVries, '83; Arai, '81; Simerly et al., '85).

In a separate series of experiments PHA-L was iontophoretically injected into the MPN. Labeled axons (Gerfen and Sawchenko, '84) were found to project from the MPN and distribute as dense plexuses with what appear to be terminal boutons in the LSv, BSTe, MeA, AVPv, VMHv and ARH. Although the MPN projects to a great many areas, the density of projections to the above sexually dimorphic regions was markedly greater than that to presumably non-sexually dimorphic regions that do not contain a high density of steroid concentrating neurons. However, structural sexual differences may exist in other nuclei such as the ventral premammillary nucleus, which also has strong bidirectional connections with the MPN, and contains a high density of steroid concentrating cells.

Taken together, these results indicate that the MPN may be a key link in a sexually differentiated neural system that is capable of responding to fluctuating levels of endogenous steroids, and underlies reproductive function, as well as other physiological mechanisms and behaviors.

357.22 AFFERENT CONNECTIONS OF THE SEXUALLY DIMORPHIC AREA IN THE PREOPTIC REGION OF THE GERBIL BRAIN. G.J. DeVries*, C.L. Gonzales* and P. Yahr. Department of Psychobiology, University of California, Irvine, CA 92717

Various mammals, including rodents, carnivores and primates, show clear sex differences in the neural structure of the preoptic area and/or hypothalamus. Often these sex differences develop under the influence of gonadal steroids. In gerbils, the sexually dimorphic area (SDA) is affected by gonadal hormones both developmentally and in adulthood. The gerbil SDA lies on either side of the third ventricle, above the suprachiasmatic nuclei and below the anterior commissure, between the medial preoptic and anterior hypothalamic areas. In males, it is hook-shaped and contains a small, dense subgroup, the SDA pars compacta (SDApc). In females, it is ovoid and lacks an SDApc. An analysis of the hormonal inputs to the SDA showed that SDA cells accumulate both androgens and estrogens and that the pattern of hormone uptake is sexually dimorphic, following the morphology of the SDA. Now we are attempting to identify neuronal inputs to the SDA.

We searched for the SDA afferents by iontophoretically applying horseradish peroxidase, conjugated to wheatgerm agglutinin, to the male SDA and its surround. One to two days later, the males were perfused, and transverse sections of the brains were reacted for peroxidase activity using tetramethylbenzidine as the chromogen. Labelled cells were conspicuous in the medial region of the bed nucleus of the stria terminalis, in the medial amygdaloid nucleus, and in the arcuate/premammillary region of the hypothalamus. Retrogradely labelled cells were also found in the ventral part of the lateral septum, in the lateral hypothalamus, and in the ventrorostral parts of the midbrain central grey. We are now attempting to determine if these areas project differentially to the various subdivisions of the SDA, e.g., to the medial versus the lateral SDA or to the SDApc. The data already available suggest that the sites projecting to the SDA are, like the SDA itself, sites that are densely labelled by gonadal steroids.

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357.23 SEROTONIN SYNAPSES IN THE MEDIAL PREOPTIC NUCLEUS (MPN) OF THE RAT: AN ULTRASTRUCTURAL STUDY. J. Larriva-Sahd*, R.A. Gorski, and P.E. Micevych (SPON: C.H. Sawyer). Dept. of Anatomy and the Laboratory of Neuroendocrinology, Brain Research Institute UCLA School of Medicine, Los Angeles, CA 90024.

There is a good correlation between the cytoarchitectonic division of the MPN and the distribution of serotonin immunoreactive fibers at the light microscopic level (Simerly et al. J. Comp. Neurol. 225:151, 1984). The lateral part of the MPN is filled with a dense plexus of serotonin immunoreactive fibers while the medial part has a low density of fibers which surround the central part of the MPN (MPNc) which is devoid of serotonin immunoreactivity. The MPNc corresponds to the sexually dimorphic nucleus of the preoptic area (SDN, Gorski et al. J. Comp. Neurol. 193:529, 1980). The sexual dimorphic distribution of serotonin immunoreactivity in the MPN may be responsible for various functions of the medial preoptic area including control of reproduction and gonadotropin release. The purpose of the present study was to examine the serotonin immunoreactivity in the MPN at the ultrastructural level. Adult male and female Sprague-Dawley rats were perfused with 4% paraformaldehyde, 0.2% glutaraldehyde in Sorensen's phosphate buffer under deep pentobarbital anesthesia. Fifty micron coronal sections obtained with a vibrating microtome were incubated with antiserum directed against serotonin (R196B, R. Elde Univ. MN), and visualized with the biotin-avidin technique using diaminobenzidine as the chromogen. The MPN was microdissected, osmicated and processed for electron microscope. Fine serotonin immunoreactive fibers (0.1-0.3 u) and boutons were localized in the MPNc as well as the medial and lateral MPN. The ratio of small (0.2-0.4 u) to large (>0.5 u) serotonin immunoreactive boutons was 10:1. The reaction product filled the dense core vesicles and was associated with the membranes of small electron lucid vesicles of the labelled terminals. The serotonin positive terminals resembled the Type I boutons of the MPNc which were classified on the basis of vesicle morphology (Larriva-Sahd and Gorski, Anat. Rec. 208:110A, 1984). These boutons are the most common type in the MPNc. The majority of the serotonin positive boutons formed symmetrical or asymmetrical junctions with varying sizes of unlabelled dentrites. These results illustrate that serotonin immunoreactive fibers and terminals are present in all three parts of the MPN although the lowest density was in the MPNc. Recently we have demonstrated that some Type I boutons contain cholecystokinin immunoreactivity, however in distinction to serotonin , cholecystokinin boutons were most dense in the MPNc. The inverse topography of the distribution of serotonin to cholecystokinin in this area may indicate a neurochemical substrate to help explain the functions of the MPN.
Supported by NS21220 and NS21060

## LIMBIC SYSTEM AND HYPOTHALAMUS II

358.1 EPILEPTIFORM BURST RESPONSES IN VENTRAL VS DORSAL HIPPOCAMPAL SLICES. M.E. Gilbert, R.J. Racine and G.K. Smith , Department of Psychology, McMaster University, Hamilton, Ontario, Canada, L8S 4K1

Field potentials from area CA1 evoked by stimulation of the Schaffer collaterals were compared in dorsal and ventral hippocampal slices of rat brain. Eighteen male Long Evans rats were anesthetized with ether, decapitated, the brain removed and the hippocampus dissected out. Five to six 450 µm slices were cut from one pole of the hippocampus perpendicular to the longitudinal axis. The stage of the tissue chopper was then rotated 180°, and 5-6 slices taken from the other end. Slices were transferred to the recording chamber where they were perfused with warmed (34.5°), oxygenated (95% $O_2$- 5% $CO_2$) artificial cerebral spinal fluid. Single pulses were delivered to the stratum radiatum through a bipolar tungsten electrode and field potentials recorded in CA1 through a glass micropipette filled with 4M NaCl under normal (3mM KCl) and increased (6mM & 9mM KCl) K+ concentrations.

Field potentials were categorized into 5 response types on the basis of their morphology, ranging from simple (single spike component) to complex (multiple spike components). A higher percentage of ventral slices (41.2%) relative to dorsal slices (2.4%) responded with a complex morphology ($X^2$=18.51, p<.001) under normal K+ conditions. Similarly, in high K+ concentrations, 90.8% of ventral and 37.4% of dorsal slices displayed multiple spike responses ($X^2$=24.47, p<.001). Thus, there is a significantly greater tendency for ventral slices to generate burst responses.

The mean amplitude of the baseline evoked response was slightly larger in ventral ($\bar{X}$=4.9 mV) than in dorsal slices ($\bar{X}$=3.5 mV), whereas the mean intensity at which maximal responses were produced was similar in the two preparations. Spike threshold was also lower for ventral ($\bar{X}$=185 µa) than for dorsal ($\bar{X}$=283 µA) slices.

Previous results have indicated a greater propensity for the development of chronic focal epileptiform discharge in ventral as opposed to dorsal hippocampal tissue Elul, R., EEG & clin. Electrophysiol. 16: 489, 1964; Racine, R. et al, Can. J. Neurol. Sci., 4: 273, 1977). This could be due to a difference in neural connections between dorsal and ventral hippocampus and other brain sites, or it may be due to differences in the internal circuitry of the hippocampus itself. The present results provide some support for the latter.

358.2 SUSTAINED HYPEREXCITABILITY RECORDED FROM THE CA3/2 AREAS OF ORGANOTYPIC HIPPOCAMPAL EXPLANTS. J. Fowler & S.M. Crain, Dept. of Neuroscience, Albert Einstein College of Medicine, Bronx, New York.

Synchronous normal & epileptiform extracellular field potentials can be recorded from diverse areas of the CNS & are particularly prominent in the hippocampal formation (Vanderwolf '69; Dichter '69). In the rodent hippocampus, extracellular sustained potential shifts occur concomitantly with hippocampal theta activity (Gerbrandt & Fowler '80) as well as during seizure discharges (Gloor et al. '61). In acute slices from adult hippocampus, epileptiform activity resembling interictal discharges in situ can be recorded in the presence of inhibitory antagonists such as penicillin whereas more complex discharges that appear transitional between interictal & ictal states are recorded in the presence of picrotoxin (Miles et al '84). Previous studies from this laboratory demonstrated that complex, long-lasting oscillatory extracellular epileptiform discharges can be evoked in organotypic explants of neocortex (Crain & Bornstein '74), spinal cord (Crain & Peterson '63) & hippocampus (Crain & Bornstein '74; Zipser et al '73; Fowler & Crain '82, '84; Fowler et al '85; see also Gahwiler '84). In the studies reported here, organotypic mouse hippocampal explants were used for the study of sustained hyperexcitability in which complex evoked slow-wave field potentials were elicited or recorded spontaneously in the CA3/2 areas after 10 days-2 months in culture. Transverse slices (.4-.9 mm thick) of mouse hippocampus (1-2 days postnatal) were explanted onto collagen-coated coverslips & incubated at 35°C in Maximow depression slide chambers. Stimuli were single pulses (0.5 msec; 5-20 uamps) applied at 8-15 min intervals. The number of stimuli needed to elicit sustained hyperexcitability was variable & could be as few as 2, or as many as 25, but typically required 4-10. After repetitive electric stimulation of the dentate area, slow- wave discharges recorded from CA3/2 became more complex and/or self-sustaining & continued to occur spontaneously for the duration of the experiment (up to 10 hrs). In the absence of inhibitory antagonists, evoked & spontaneous oscillatory epileptiform discharges were recorded over a wide range of extracellular $K^+$ concentrations (3-9 mM). These discharge patterns were generally more complex than the interictal-like discharges recorded in the acute adult hippocampal slice in the presence of penicillin but similar to those recorded in the presence of 100 uM picrotoxin (Miles et al '84; Traub et al '84), although the interval between afterdischarges as well as the duration of the discharge sequence was often greater than reported in these studies. Continuous epileptiform discharges similar to those observed during ictal events were not elicited with this stimulation paradigm, although preliminary tests indicate that perfusion with 4-aminopyridine can more closely approximate ictal events. Supported by NIMH grant #15788. Tissue culture facilities were kindly provided by Dr. Murray B. Bornstein.

358.3 MODELS OF PROPAGATION OF SYNCHRONIZED EPILEPTIFORM EVENTS IN THE HIPPOCAMPAL SLICE. Roger D. Traub, W. Douglas Knowles, R. Miles and R.K.S. Wong. IBM T.J. Watson Research Center, Yorktown Heights, NY 10598 and Dept. of Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX 77550.

We have shown (Knowles, Strowbridge and Traub, this meeting) that spontaneous epileptiform activity, as recorded by initial deflection of extracellular field potentials, propagates across the CA2-CA3 region of the convulsant-treated hippocampal slice at velocities of about 0.1 m/s. In the present study, we used computer simulations of 10x50 networks of model neurons to examine possible mechanisms underlying this propagation. We considered two different anatomical cases: (1) synaptic connections are sparse, but those connections which do exist are powerful enough to allow bursting to propagate from presynaptic cell to postsynaptic cell; (2) every cell has synaptic connections to many other cells, but synaptic connection strengths are not all identical but rather are distributed (independent of intercellular distance) so that only a minority of connections are powerful enough to allow burst propagation. We did not consider the case where each cell has a powerful connection to many other cells, since dual intracellular recordings only rarely demonstrate a functional excitatory connection between neurons. In case (1) above, random networks do not exhibit propagation of epileptiform field potentials, even when axonal conduction velocity is as slow as 0.05 m/s. For propagation to occur in this case, connections must occur preferentially to nearby cells. A time-consuming local integration process, which is slow relative to conduction time, is necessary before field potentials are generated. However, in case (2), propagation of the epileptiform activity can occur with sufficiently slow axon conduction velocity (0.2 m/s). Activity initiated in a few cells has subliminal as well as suprathreshold effects on follower cells. The subliminal fringe initially consists of nearby cells and grows with time, depending on conduction velocity. Strong connections can cause cells within the subliminal fringe to fire, generating additional subthreshold excitation. In this way, an orderly spatial recruitment of neurons arises within the network, despite a random connectivity.

358.4 INITIATION AND SPREAD OF EPILEPTIFORM BURSTING IN THE HIPPOCAMPAL SLICE. W.D. Knowles, B.W. Strowbridge* and R.D. Traub. IBM Thomas J. Watson Research Center, Yorktown Heights, NY 10598.

Convulsant treated hippocampal slices produce spontaneous, synchronized epileptiform bursts of neuronal activity which have been suggested to originate in the region of CA2 (Wong & Traub, J. Neurophysiol. 49:442-458, 1983). It is unknown whether there is a small pacemaker focus from which bursts originate or whether bursts start as a more diffuse buildup of activity over a larger population of neurons. Further, it is unknown whether subsequent spontaneous bursts originate from the same site, or from different, fluctuating sites. We addressed these questions in this study.

We measured field potentials generated by picrotoxin and penicillin induced epileptiform activity in the hippocampal slice. We used an array of 16 electrodes made from fine wires inserted through electron microscope grids in a curved pattern to match the curvature of CA2/3 stratum pyramidale.

Spontaneous epileptiform bursts from any given slice were very stereotyped; there was little variation from burst to burst in the pattern of potentials recorded. Variations from slice to slice were much larger. Penicillin and picrotoxin produced similar results. Spontaneous bursts always appeared first at electrodes near CA2, then spread smoothly and uniformly away from the site of origin. The velocity of propagation was 0.12 ± 0.05 m/sec (mean ± S.D.; n=13 slices). Occasionally, we could observe small increases in baseline noise preceding the onset of spontaneous epileptiform bursts in electrodes at which the burst first appeared. Picrotoxin induced afterdischarges originated at the same electrode site and propagated with similar velocities as the primary burst.

The pattern of origin and spread of bursts evoked by suprathreshold stimulation of mossy fibers or Schaffer collaterals was substantially different than spontaneous bursts. Mossy fiber and Schaffer collateral evoked bursts originated in CA3c and CA3a respectively, and spread two to three times faster than spontaneous bursts. The velocity of spread of stimulus evoked bursts was similar to conduction velocities of mossy fibers and Schaffer collaterals measured in normal medium using the electrode array.

These results demonstrate that spontaneous synchronized epileptiform bursts originate from a constant, well-localized focus which is near, if not identical with, CA2. The bursts spread smoothly across CA3 in a constant pattern with a velocity of about 0.1 meter/sec. This is slower than the conduction velocity of the major axons in this area. It appears that there is some low level of activity at the focus of origin immediately preceding the onset of the burst. These findings place stringent constraints on the possible patterns of local synaptic connectivities and mechanisms in this area (see accompanying paper by Traub et al.).

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358.5 INHIBITION INDUCED BY ELECTRICAL STIMULATION IN SYNCHRONOUSLY FIRING HIPPOCAMPAL NEURONS IN MAN. M. Isokawa-Akesson, T.L.Babb and C.L.Wilson. Brain Research Institute and Dept. of Neurology, School of Med., UCLA, CA 90024.

A synchronized firing of neurons raises the question of how the excitability and the timing of these neurons are temporarily yoked and controlled by a mechanism different from that of independently-firing neurons. Previous findings showed that limbic neurons tend to fire synchronously, with longer inter-spike-intervals, in temporal lobe epilepsy patients (Isokawa-Akesson, et al. Neurosci. Abs. 1984.p188). The present study was designed to test durations of neuronal inhibition induced by nearby electrical stimulation. Anterior hippocampal(AHP) regions were electrically stimulated with single biphasic pulses every 10 sec (pulse duration: 100 μsec for each direction, 40 pulses, 1.5-3.0 mA). AHP neurons within 5-10 mm of the stimulation were monitored extracellularly for excitatory and inhibitory patterns. All neurons were initially tested, before the stimulation, for synchronized firings with other AHP neurons by cross-correlation analysis, with an analysis time of 50 msec before and after the expected joint firing.

Local AHP stimulation caused initial excitation in some of AHP neurons with a latency of 10.7 msec + 3.43 SEM followed by inhibition of firing. The duration of this inhibition correlated with whether or not AHP neurons had synchronized firing with other AHP neurons. In synchronously-firing AHP neurons(SAHP), stimulus-evoked inhibition averaged 412.4 + 37.81 msec. In contrast, AHP neurons that fired without synchrony to other AHP neurons(NSAHP) had average inhibitory duration of 83.0 + 18.2 msec. A second difference between SAHP and NSAHP neurons was the change in firing rate. The long recurrent inhibition induced by local AHP stimulus volleys to SAHP neurons caused a 62.4% decrease in average firing rate from its spontaneous rate. In contrast, in NSAHP neurons, stimulus volleys actually increased the average firing rate by 119.2% above the average spontaneous rate.

This finding, i.e., 1) stimulus evoked duration of inhibition in AHP neurons are longer if these neurons spontaneously fire in synchrony with other AHP neurons, and 2) the average firing rate of synchronized AHP neurons decreased when these neurons were stimulated, supports the model by Finch and Babb(Brain Res. 354. 1977) that this inhibition has a synaptic component and is determined by a local recurrent GABA-mediated inhibition. A synchronous firing of two AHP neurons may engage basket-cell-inhibition with temporal summation and produce prolonged inhibition. The functional significance of this inhibition in synchronously-firing neurons is proposed as a mechanism for setting the timing for AHP neurons to fire synchronously.

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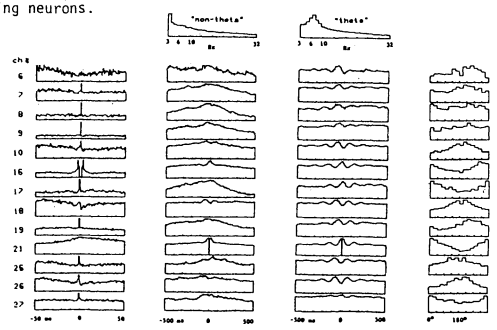
358.6 GROUP DYNAMICS OF HIPPOCAMPAL NEURONS: PATTERNS OF UNIT ACTIVITY DURING THE THETA RHYTHM. M. Kuperstein, T. VanDemark*, and H. Eichenbaum Dept. Biol. Wellesley College, Wellesley, MA 02181

Using a 24-channel microelectrode system called PRONG (Parallel Recording of Neural Groups; Neurosci. Abstr. 10:598), we have begun a study of hippocampal neural group activity by simultaneously observing the activity of several single units during theta and non-theta defined behavioral states. In several rats, up to 14 single units in the flexure of CA3 were simultaneously monitored. The hippocampal EEG was recorded across the contralateral CA1 pyramidal cell layer.

Units were analyzed by a combination of methods: inspection of spike waveform, computation of firing rate and phase relation to theta autocorrelation, and cross-correlation of spike events. Examples of correlograms are shown below (left panel synch on channel 16 at 0ms; next two panels synch on channel 21; each correlation individually normalized). Short-term firing patterns were relatively constant acro theta and non-theta states, whereas longer-term neural group patterns were selective to the theta state. At high time resolution (1ms/bin; left panel), we frequently observed bursting repetoires, as shown in their autocorrelation (e.g. channel 16), and co-activation peaks near 0ms. The degree of bursting and co-activation was similar across theta and non-theta states. At a lower time resolution (10ms/bin), cross-correlation during non-theta (second panel, power spectrum at top) indicated only broad periods of co-activation. In striking contrast, the same neurons became synchronously active during theta activity (third panel), even though these cells had different preferred phase relations to the ongoing theta cycle (right panel). The combined findings suggest that the theta rhythm signifies a powerful patterning of group activity imposed on a fixed connectivity of neighboring neurons.




(Supported by HHS NS 18744 & ONR 00014-83-K0337)

358.7 MEMBRANE PROPERTIES OF AMYGDALA NEURONS AND THE EFFECT OF ESTROGEN. J. Nabekura*, Y. Oomura, Y. Mizuno* and T. Minami* (SPON: M. Tachibana). Dept. of Physiol., Fac. of Med., Kyushu Univ. 60, Fukuoka 812, Japan.

The amygdaloid body, which has many nuclei, is important in emotional behavior. The medial (MD) and basolateral (BL) nuclei affect behavior, such as feeding, aggression and sex, in opposite ways. Intracellular recordings were obtained from MD and BL neurons of rat brain slices in vitro, and effects of estrogen, which modulates feeding and sexual behavior, were examined in male and female rats.

Application of outward current pulses to the cell membrane normally induced only repetitive Na spikes in all MD neurons, but Ca spikes appeared only in TTX containing medium. These Ca spikes were blocked by external Co. Under normal condition both Na and Ca spike complexes were observed in 23% of BL neurons. The results indicate that the BL nucleus contains neurons with a type of firing which is different from that of MD neurons. The afterhyperpotentials (a.h.p.) that followed the spikes had a reversal potential of -93mV. The a.h.p. disappeared upon application of TEA and 4-aminopyridine (4-AP),($E_K$= -93mV). Delayed return of the membrane potential to resting level after cessation of an inward current pulse was blocked by 4-AP. This indicated an increase of K ($I_A$) conductance and occurred in both MD and BL neurons.

Stria terminalis stimulation elicited EPSP-IPSP sequences in 90% of the MD and 82% of the BL neurons. Application of bicuculline eliminated the IPSP with a reversal potential of -64mV ($E_{Cl}$= -64mV). External capsule stimulation elicited only EPSPs in 64% of the BL neurons.

The MD nucleus is well known to contain many estrogen receptors and differs histologically in the two sexes. Hyperpolarization of approximately 15mV accompanied by an increase in membrane conductance was induced by $10^{-8}$M 17β estradiol hemisuccinate (17β$E_2$S) in 23% of the MD neurons during normal estrous cycle or in ovariectomized estrogen-primed female rats. This effect persisted even after application of TTX and Cd. The effect of 17β$E_2$S on membrane potential and determination of the membrane I-V relationships with and without 17β$E_2$S indicated a reversal potential for the 17β$E_2$S hyperpolarization of about -90mV. This could be changed by altering K concentration in the medium, which indicates that 17β$E_2$S directly increases the K conductance of MD neurons. On the other hand, 17β$E_2$S had very little effect on male or female BL neurons, and induced responses in only 8% of male MD neurons. These sexual and regional membrane characteristics, inputs, and drug effects might be related to functional and morphological differences between MD and BL neurons, and between male and female MD neurons.

358.8 PHYSIOLOGICAL PROPERTIES OF IN-VITRO RAT MEDIAL SEPTAL NEURONS. M. Segal* (SPON: A. Mayevsky). Center for Neuroscience, The Weizmann Institute of Science, 76100 Rehovot, Israel

Medial septal neurons fire rhythmic bursts of action potentials at characteristic high rates in vivo. Activity of these neurons is associated with the generation of hippocampal theta rhythm; there is a good correlation between septal cellular activity and theta waves, stimulation of the medial septal area (MS) generates hippocampal theta and lesion of the MS abolishes it. The mechanisms underlying the rhythmic septal activity are unknown.

I have studied MS neurons in a slice taken from adult rat brain in an attempt to detect and analyze some physiological properties of these neurons that might mediate their rhythmic activity. Most MS neurons exhibit strong non-linearity in the IV relations on both the depo- and hyperpolarizing sides of the resting potential. A large proportion of MS neurons (over 1/4 of the cells) discharge spontaneously in vitro. The other neurons may discharge rhythmically upon depolarization by passage of constant current pulses across the recorded cell membrane. Rhythmicity is preserved when $Na^+$ action potentials are blocked by TTX, provided the cell is loaded with $Cs^+$ to block K currents. Rhythmicity disappears in the presence of Ca antagonists $Cd^{2+}$ or $Co^{2+}$. Also, rhythmicity disappears by hyperpolarizing the cell, indicating the absence of an underlying synaptic mechanism. Several phenomena might be related to the rhythmic activity of MS neurons: 1. a large depolarizing after potential (DAP) commonly results in firing of a second action potential at constant (4-5ms) latency after a first one. 2. a rebound depolarization seen after termination of a hyperpolarizing current pulse may occasionally fire an anodal break potential. 3. Lack of significant accomodation of the firing frequency in response to a depolarizing current pulse. MS neurons receive an inhibitory input from the lateral septal area (LS). GABA antagonists block the response to LS stimulation as well as spontaneous PSP's which share the same reversal potential as that seen in response to GABA. These results indicate that rhythmicity of in vivo MS cells is probably intrinsic and is only modulated by synaptic inputs.

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358.9 STABILITY OF CORTICAL EEG ACTIVITY DURING SENSORY INTERACTION. L.C. Oatman. U.S. Army Human Engineering Lab., Aberdeen Proving Ground, MD 21005

During sensory interaction, an accessory stimulus in a second modality can have a facilitating effect upon some measurable response to a stimulus applied to a primary modality. Since sensory stimulation can elicit hippocampal rhythmic slow activity (RSA) during behavioral immobility, this study examined whether increased intensity of an accessory light stimulus increased hippocampal RSA (theta waves) during behavioral immobility in a sensory interaction paradigm.

Cortical EEG activity was recorded from six chronically implanted cats when auditory click stimuli were presented before, during, and after simultaneous accessory light stimulation of seven different intensities. Sample epochs of auditory cortex EEG activity from the three recording sessions, in the absence of bodily movement, were subjected to the fast Fourier transform, and the average power spectra were determined for the frequency bands theta (4-8 Hz), alpha (8-13 Hz), beta 1 (13-20 Hz), and beta 2 (20-40 Hz). The amount of power within the theta frequency range was not significantly different during simultaneous visual and auditory stimulation when compared with the pretest and posttest control periods of auditory stimulation, except at the most intense level of light stimulation. Simultaneous auditory and visual stimulation had no systematic effect on the amount of power occurring in the alpha, beta 1, and beta 2 frequency bands. The results suggest that sensory interaction effects do not produce hippocampal RSA (theta) during behavioral immobility when both the auditory and visual stimuli are irrelevant, except at very intense levels of stimulation.

358.10 LOCATION-SPECIFIC FIRING OF HIPPOCAMPAL THETA CELLS. J.L. Kubie, L. Kramer* and R.U. Muller*. Dept. of Physiology, Downstate Medical Center, Brooklyn, N.Y., 11203.

Complex-spike (CS) cells recorded from the hippocampus of freely moving rats fire fast only when the rat is in a delimited portion of its environment. Outside the region of rapid firing (the firing field), CS cells are virtually silent. CS cells thus have extremely clear spatial firing patterns; the in-field to out-of-field firing rate ratio is about 50:1 or more. Theta cells, which are local circuit neurons, can also be recorded from the hippocampus. They are identified by the fact that their firing rate increases 2-fold or more whenever the hippocampal EEG is in the "theta" state. Since theta cells are excited by CS cells, some degree of location-specific firing ought to be apparent in theta cells. We cannot, however, expect to see obvious firing fields, since theta cells are never silent in freely moving rats.

The techniques are the same as those previously used to study CS cells. During 16 minute sessions, rats chase food pellets dropped on the floor of a familiar cylindrical enclosure. The rat is located in a 64 by 64 matrix at 60 Hx; the number of spikes fired by the target cell during each 1/60th sec. is counted. Subsequently, color-coded firing rate maps are used to display the spatial firing pattern.

We have recorded from 9 theta cells. All showed spatial coherence of firing as judged by eye; that is, their firing rate maps were not uniform. Continuous regions of approximately constant firing rate were seen. The firing rate in "hot regions" was about twice that in slow firing regions. Such regions, whether associated with rapid or slow firing were quite large. Since the continuous regions taken together fill the apparatus, their approximate size can be estimated by noting that there were between two and 5 such regions in the maps for our sample. Crucially, the spatial firing patterns seen for individual cells were reproducible from session to session, as determined by eye and by pixel-by-pixel correlations between the spatial firing rate distributions.

Two further observations have been made about the spatial firing properties of theta cells. For three cells, we sorted samples into 4 categories according to the concurrent state of the hippocampal EEG. These categories ranged from strong theta to large, irregular activity (Vanderwolf, Electr. clin. Neuro., 1969). The spatial patterns obvserved for each category were quite similar to each other. Thus, the state of the hippocampal EEG is not the only correlate of theta cells activity. Second, on two occasions, we looked to see if spatial fring patterns differ among theta cells in the same rat. Ideally, two theta cells would be recorded simultaneously. Due to current technical limitations, we instead alternated sessions for each of two cells. The spatial firing patterns as judged by eye were very different; moreover, pixel by pixel correlations between sessions for the same cell were high, whereas similar correlations across cells were low. Thus, spatial firing patterns for theta cells are cell-specific. This reinforces our notions that theta cells are activated by particular sets of CS cells.

358.11 UNIT ACTIVITY IN THE MONKEY HIPPOCAMPAL REGION
M.W. BROWN* & F.A.W. WILSON (SPON: B. Matthews), Department of Anatomy, The Medical School, University of Bristol, Bristol BS8 1TD, U.K.

In previous experiments hippocampal unit activity has been found to be dependent on the context as defined by the behavioural task within which the stimuli were presented (1). Monkeys performed a delayed match to sample task: a trial consisted of 2 successively presented stimuli (S1 and S2) on a video monitor. Fruit juice was obtained by a right panel press when both stimuli were the same, or a left press when the stimuli differed. Standard stimuli were squares and circles. Context-dependent unit activity was selectively related to a specific stimulus (e.g. a large square) as S2 on a particular type of trial (match or non-match).

Recent experiments have examined the activity of hippocampal region units to familiar, novel and not recently seen stimuli in both the delayed matching task and in other tasks in which junk objects and foods could be viewed, or viewed and obtained by the monkey. Further examples of units with context-dependent responses and units selectively responsive to specific stimuli have been found. Units responsive in the delayed matching task were often also responsive to junk objects and foods. Some units responded maximally to novel or not recently seen stimuli, with rapid habituation to repeated stimulus presentations. These responses indicate access to both direct and stored sensory information.

Task-related unit activity often also occurred after the offset of the visual stimuli, in the interval between trials, and in some cases was dependent on the animal's motor response (right or left press). However, neuronal activity was not solely determined by mouth, arm and eye movements. Thus activity of hippocampal units codes both sensory and motor aspects of the behavioural tasks. These data support a role for the primate hippocampal region in the sensory-motor integration of learned behaviour.

(1) M.W. Brown (1982) in "Neuronal Plasticity and Memory Formation", Ed. C. Ajmone Marsan & H. Matthies pp. 557-573.

358.12 EVIDENCE FOR LOCAL EXCITATORY SYNAPTIC CIRCUITS IN CA1 OF RAT HIPPOCAMPAL SLICES OBTAINED BY GLUTAMATE MICROSTIMULATION.
E.P. Christian* and F.E. Dudek (SPON: D. Holtzman). Dept. of Physiol., Tulane Univ. Sch. of Med., New Orleans, LA 70112.

Recurrent axon collaterals of hippocampal CA1 pyramidal cells, which terminate within CA1, could hypothetically subserve both excitatory and inhibitory local synaptic interactions. A direct physiological investigation with dual intracellular recordings from hippocampal slices cut in a longitudinal orientation only provided evidence for inhibitory local synaptic circuits between CA1 pyramidal cells (Knowles and Schwartzkroin, J. Neurosci., 1:318, 1981). It is possible, however, that local excitatory connections were not detected in this study because of technical problems; that is, the interactions could be rare or not oriented in a longitudinal plane. We reinvestigated this issue in hippocampal slices cut in two orientations by stimulating neuronal populations in CA1 with glutamate (Glu) microdrops. Increases in the frequency of spontaneous excitatory or inhibitory postsynaptic potentials (EPSPs or IPSPs) in a nearby CA1 pyramidal cell were used as evidence for local excitatory or inhibitory connections.

Rat hippocampal slices (450 µm thick) were cut either parallel (transverse plane) or perpendicular (longitudinal plane) to alvear fibers and maintained using standard procedures. Pyramidal cells in CA1 were recorded intracellularly while microdrops (10-50 µm dia.) of Glu (10-20 mM) or control saline were applied at several places in the CA1 stratum pyramidale at distances ranging from 0-400 µm on either side of the recording site. Spontaneous EPSPs and IPSPs (>3 mV) were counted for 10-sec periods before and after each microdrop application.

Glutamate microapplications in at least one location in CA1 consistently increased the frequency of EPSPs in 6 of 9 CA1 pyramidal cells tested in transverse slices. In longitudinal slices, glutamate microapplications did not alter the EPSP frequency in any CA1 pyramidal cells (n=10). Slow hyperpolarizations and/or increases in IPSPs were observed following glutamate applications in some cells from both transverse and longitudinal slices.

Our data support evidence from previous dual intracellular recording studies that local inhibitory circuits are present in CA1 of longitudinal hippocampal slices. They further suggest that local excitatory circuits exist in CA1, and that they are oriented predominantly in a transverse plane running parallel to the alveus. Although dual intracellular recordings will be necessary to confirm these data, the possible presence of local excitatory connections in CA1 should be considered in models of epileptiform synchronization in this hippocampal area.

Supported by NIH grant NS 16683.

358.13 SILENT HIPPOCAMPAL PYRAMIDAL CELLS IN AWAKE FREELY BEHAVING RATS.
L.T. Thompson,* J. Kapur,* P.N. Lacey,* R.A. Salerno,* & P.J. Best, University of Virginia, Charlottesville, VA 22901

Over the past 10 years, we noted many extracellularly recorded hippocampal pyramidal cells isolated in anesthetized rats either were lost or became silent when the animals recovered from anesthesia. Kubie & Ranck (1982) have reported many cells which show place field activity in one environment are silent in others. Most of our silent cells showed robust complex-spike activity when the rats were re-anesthetized. The cells were not lost, but apparently were inhibited from firing during our experiments in the awake animal.

The present study quantifies and describes the characteristics of silent cells. Ten rats were implanted with moveable bundles of ten 32 um nichrome microwire electrodes. The rats were later anesthetized with 30-40 mg/kg of pentobarbitol followed by 0.3 mg/kg of atropine sulphate, and one or more cells were isolated with 4:1 signal-to-noise ratios. Unit activity was recorded while the rats were anesthetized, after waking from anesthesia, on a radial arm maze, in a cylindrical drum, in a shock box during classical conditioning and during subsequent re-anesthetization. Automatic registration of the rat's location was combined with on-line computer analysis of cell firing to analyze place field activity.

Of 26 dorsal hippocampal cells isolated under anesthesia, 15 were silent upon awakening. The remaining 11 cells showed place field activity in at least one of the three testing environments. Five cells had active place fields in more than one environment. During final anesthetization, 10 of the 15 cells which were silent in the awake rat again showed robust activity, as did all 11 place cells.

Powerful inhibition of hippocampal pyramidal cell firing has been shown in our lab during REM sleep and upon arousal from slow wave sleep. This inhibition may enhance the processing of multisensory spatial information which occurs within the hippocampus. The rapid firing of a place cell when a rat is physically within the place field appears to be actively inhibited when the rat is outside that field. Information is thus expressed both by those cells which are active within a particular location, and by those which are silent.

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358.14 DOES A REDUCTION IN RECURRENT INHIBITION CONTRIBUTE TO PAIRED PULSE POTENTIATION OF THE CA1 POPULATION SPIKE IN THE HIPPOCAMPUS?
M.W. Oliver* and J.J. Miller (SPON: J.A. Pearson), Dept. of Physiology, University of B.C., Vancouver, B.C., Canada V6T 1W5

A recent study (Oliver and Miller, Can. J. Physiol. Pharmacol., 1985) showed that repetitive stimulation of the alveus reduced the recurrent IPSP in hippocampal CA1 pyramidal cells. This effect could be observed on the second pulse of the stimulus train suggesting that the CA1 pyramidal cell's IPSP may be diminished by a paired pulse (PP) paradigm. Thus, the possibility exists that potentiation of the population spike (PS) associated with paired pulse stimulation of the stratum radiatum (SR) is in part due an increased excitability of pyramidal cells resulting from an attenuation of the IPSP. Therefore the present study was undertaken to examine the contribution which a reduction in recurrent inhibitory processes may have in the PP potentiation of the population spikes recorded from the CA1 area of rat hippocampal slices.

Paired homosynaptic stimulation of SR results in the characteristic inhibition-potentiation of the PS at condition-test (C-T) intervals < 0.04s and 0.06-8 s, respectively. In contrast to these data, paired heterosynaptic SR stimulation resulted in the inhibition of the test PS at all C-T intervals. In order to assess the effect of a SR conditioning pulse on the recurent inhibition, the alveus was stimulated 20 ms before the test SR pulse. With either homo- or heterosynaptic stimulation the magnitude of the recurrent inhibition was enhanced for C-T intervals of up to 0.1 s; however, at all other intervals tested (< 8s), the inhibition was significantly attenuated.

Intracellular recordings obtained from CA1 pyramidal cells indicate: 1) the PP inhibition observed in response to heterosynaptic stimulation correlates with the time course of the afterhyperpolarization (AHP); and 2) a significant reduction in the amplitude and conductance of the alvear-evoked IPSP occurs during the AHP.

These data support the suggestion that the PP potentiation phenomenon in CA1 not only involves perisynaptic processes but also postsynaptic mechanisms, particularly those mediating pyramidal cell inhibition.

358.15 DIFFERENTIAL SENSITIVITY OF RAT AND GUINEA PIG HIPPOCAMPAL PYRAMIDAL NEURONS TO CALCIUM-DEPENDENT PROCESSES: BIOCHEMICAL AND ELECTROPHYSIOLOGICAL EVIDENCE. P. Leung*, J.J. Miller and K.G. Baimbridge, (SPON: P.C.K. Leung), Dept. of Physiology, UBC, Vancouver, B.C., Canada. V6T 1W5

Previous immunocytochemical studies have demonstrated that the hippocampal pyramidal cells in the CA1 region and granule cells in the dentate gyrus contain calcium-binding protein (CaBP). This localization is characteristic of most species (rat, mouse and monkey), with the exception of the guinea pig in which CaBP is not present in the CA1 pyramidal cells (Baimbridge and Miller, this volume). Such a species difference for a single population of neurons suggests the possibility that these cells may also exhibit significant differences in their electrophysiological properties. We have therefore compared the responses of CA1 pyramidal neurons in the rat and guinea pig hippocampal slice preparations following perfusion with 1) low calcium medium (0.1mM $Ca^{2+}$, 2.0mM $Mg^{2+}$) to determine the sensitivity of these preparations to low calcium induced bursting; 2) pulse application of a high calcium medium (4mM $Ca^{2+}$, 1.0mM $Mg^{2+}$) which we have previously shown to induce long-term potentiation (LTP) in the rat and 3) picrotoxin (10 μM).

In the presence of a low calcium medium the CA1 pyramidal neurons of the rat exhibited the characteristic spontaneous burst activity which persisted for hours. In comparison, the onset of bursting in the guinea pig preparation was significantly delayed from that of the rat and its frequency considerably slower. Pulse application of a 4mM $Ca^{2+}$ medium (4-10 min) successfully induced LTP of the stratum radiatum (SR) evoked EPSP and population spike in the CA1 region of the rat but was ineffective in the guinea pig. In this latter preparation a subsequent tetanic stimulation of the SR induced a robust LTP. Perfusion with picrotoxin resulted in multiple population spikes evoked by stimulation of the SR in both species but was far more effective in inducing spontaneous interictal burst activity in the guinea pig than in the rat in terms of onset latency and frequency.

These observed differences of rat and guinea pig CA1 neurons to processes underlying burst discharge generation and potentiation phenomena may well be related to the presence or absence of CaBP, which has been suggested to play a role in the intracellular regulation of calcium ions. In any event these data suggest that the rat and guinea pig CA1 pyramidal cells exhibit clear differences in both biochemical and electrophysiological properties. Such differences may be important factors to take into consideration when comparing reports from different laboratories using either of these two species. In addition, a comparison of the properties of the CA1 pyramidal cells from the rat and guinea pig may help to reveal the possible function(s) of CaBP in the central nervous system.

358.16 COMPARATIVE DISTRIBUTION OF CALCIUM-BINDING PROTEIN AND PARVALBUMIN IN THE RAT, MOUSE, GUINEA PIG AND MONKEY HIPPOCAMPAL FORMATION. K.G. Baimbridge and J.J. Miller, Dept. of Physiology, UBC, Vancouver, B.C., Canada V6T 1W5

Calcium binding protein (CaBP) and parvalbumin (PV), two members of a family of proteins which bind calcium ions with high affinity, have been shown to be present throughout the cytoplasmic volume of specific neuronal populations within the central nervous system. We have previously reported that in the hippocampal formation of the rat, CaBP is present in two of the major cell types (CA1 pyramidal and dentate granule cells) but is absent from the CA3 pyramidal neurons. In the present study the distribution of this protein in the hippocampal formation of a number of different species was examined. In addition its topographical localization was compared to that of PV.

Antibodies to human cerebellar CaBP and mouse muscle PV were raised in rabbits and used to localize these proteins in cryostat sections (20 μ) of the hippocampal formation of rat, mouse, guinea pig and monkey brains fixed by formalin perfusion. The methodology has been previously described (Baimbridge and Miller 1982, Brain Res. 245, 223-229).

The marked differential distribution of CaBP previously reported for the rat was confirmed and an identical distribution was found in the hippocampal formation of the mouse and monkey. In the guinea pig, however, CaBP was found only in dentate granule cells. The absence of CaBP in the CA1 pyramidal cells may be functionally associated with electrophysiological differences observed in hippocampal slice preparations taken from either the guinea pig or rat, (see Leung, Miller and Baimbridge, this volume).

The localization of PV immunoreactivity was quite distinct from that of CaBP but was similar in all species examined. In the CA1 region a heavily stained population of small multipolar cells having short dendritic processes were present in the stratum oriens and alveus. Positively labelled neurons were also scattered throughout the stratum pyramidale of the CA1-CA4 regions and their processes extended along the cell body layer as well as into the stratum radiatum. A similar topographical localization was observed in the dentate gyrus where the stained cells were located mainly in the basal portions of the granule cell layer and their dendrites extending vertically into the stratum moleculare. Both granule and pyramidal cell layers contained a dense plexus of positively labelled fibers/terminals. While PV immunoreactive cells may represent a subpopulation of principal cells within the hippocampal formation which are distinct from those labelled by CaBP, their distribution and morphological characteristics would appear more closely associated with local circuit neurons, particularly those which have been classically identified as basket cells.

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358.17 HIPPOCAMPAL CALCIUM REGULATION AS MEASURED BY KINETIC ANALYSIS OF $^{45}Ca$ UPTAKE CURVES IN THE SLICE PREPARATION. I. Mody and J.J. Miller, Dept. of Physiology, University of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

Kinetic analysis of $^{45}Ca$ uptake curves has been widely used in several non-neuronal preparations to examine intracellular $Ca^{2+}$ compartmentalization. The aim of the present study was to apply this technique to the hippocampal slice preparation in view of the variety of calcium-mediated phenomena shown to exist in this CNS structure. The method consists of introducing the tracer into the extracellular medium and monitoring its uptake into the cellular network. The uptake curve is then fitted by a mathematical function and the ionic fluxes, rate constants and distributions are determined according to the principles of compartmental analysis.

The hippocampal $^{45}Ca$ uptake curves were fitted using the non-linear least squares Simplex method and were found to be best approximated by a double exponential equation. This finding indicates that the slice preparation behaves as having two exchangeable pools of $Ca^{2+}$. Both compartments are localized intracellularly since the bulk of extracellular $Ca^{2+}$ has been washed off by $La^{3+}$ following termination of the uptake.

The first compartment is characterized by a rapid rate of exchange ($T_c$=3.75 min) and its size increases linearly with alterations in $[Ca^{2+}]_o$ from 0.1-4.0 mM. Therefore it most likely represents the sum of $Ca^{2+}$ bound to the plasma membrane and free ionic intracellular $Ca^{2+}$. The second compartment has a slower exchange rate ($T_c$=33.33 min) and is readily altered by incubation of the slices with the mitochondrial uncoupler DNP and the phosphodiesterase inhibitor IBMX ($10^{-4}$M). This compartment is considered to include buffered/sequestered intracellular $Ca^{2+}$.

Contrary to expectations, the $Ca^{2+}$-channel antagonist nifedipine ($10^{-6}$M) did not block the uptake of calcium into the rapidly exchangeable pool, but instead caused an enhancement of the buffered pool. Salmon calcitonin (4 U) effectively reduced the total exchangeable $Ca^{2+}$ of the hippocampal slice through mobilization of the cation from intracellular storage sites while increasing $Ca^{2+}$ efflux.

Changes in $Ca^{2+}$-regulation as measured by the kinetic analysis were also reflected in altered electrophysiological characteristics of hippocampal neurons. It is concluded that although the hippocampal slice preparation is a heterogeneous system consisting of various types of neurons and glial cells it may nevertheless be characterized with regard to its $Ca^{2+}$-homeostasis using the kinetic analysis of $^{45}Ca$ uptake. The method provides a novel approach to investigation of CNS calcium regulation under both physiological and pathophysiological conditions.

359.1 EFFECTS OF COBALT-INDUCED CHRONIC AMYGDALOID SEIZURES UPON AFFECTIVE DEFENSE BEHAVIOR IN THE CAT. M.B, Shaikh*, M. Brutus, H. Edinger, and A. Siegel. Depts. of Neuroscience and Physiology, University of Medicine and Dentistry of New Jersey -- N.J. Medical School, Newark, N.J. 07103.

Studies conducted previously in our laboratory (Block et al, 1980, Siegel, 1984) and elsewhere (Stoddard-Apter and MacDonnell, 1980) have demonstrated that amygdaloid nuclei can differentially modulate affective defense and quiet biting attack behavior elicited from the hypothalamus of the cat. More recently, our laboratory has further demonstrated that seizures induced acutely by electrical stimulation of the amygdala and pyriform cortex can also signficantly alter thresholds for each of these responses (Brutus et al, 1984). The present study was conducted in order to identify the effects of seizures chronically induced in the amygdala by metallic cobalt powder upon affective defense behavior.

Monopolar electrodes and chemtrodes for stimulation and recording were stereotaxically implanted into the medial and lateral aspects of the hypothalamus, amygdala and pyriform cortex. Initially, stable baseline thresholds for affective defense were determined over a 1-3 week period utilizing an ascending and descending Method of Limits. Then, cobalt (approximately 50 mg.) was administered through a chemtrode into the amygdala or pyriform cortex in order to induce chronic seizures. Following administration of cobalt, baseline attack thresholds were again determined on alternate days over a 4 week period. At the completion of the experiments, animals were sacrificed and histological localization of electrode and chemtrode tracks were determined.

Our results have suggested that the effects of chronic seizures upon affective defense behavior are largely dependent upon the amygdaloid and pyriform cortical sites involved in the primary cobalt focus. In 4 cases, animals sustaining chronic seizures in which a primary focus involved the pyriform cortex showed a lowering of response thresholds over time. In contrast, animals whose primary seizure focus included the lateral and/or central nucleus displayed an increase in attack response thresholds over time. Alterations in attack thresholds also appeared to be dependent upon seizure intensity.

These findings suggest the following conclusions: (1) that chronic amygdaloid and pyriform cortical seizures can significantly modify hypothalamically-induced affective defense behavior, and (2) that the directionality of effects is specific to the location of the seizure focus.

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359.2 CAT "P300": EFFECTS OF SEPTAL LESIONS. J. Harrison, J. Buchwald, K. Kaga,* N. Woolf and L. Butcher. Depts. of Physiology and Psychology, Brain Res. Ctr. Ment. Ret. Res. Inst., UCLA, Los Angeles, CA 90024.

We have previously reported on a cat model of the P300 and are now engaged in a series of studies to elucidate the generator substrates of this endogenous cognitive potential. Because the hippocampus is a postulated generator, in this study we lesioned the septal nuclei and nuclei of the diagonal band to interfere with the normal cholinergic innervation of the hippocampus. The object of this experiment was to determine whether the septal lesion depleted hippocampal acetylcholinesterase (AChE) and significantly altered the vertex recorded endogenous response. Responses were recorded for 12 pre- and 12- post-operative sessions for each cat. Acoustic stimuli included loud clicks which were rare (15%) or frequent (80%); soft clicks which were reciprocally frequent or rare, and tone pulses (5%) followed by eyelid shock which focussed the cat's attention. EEG recorded from a steel screw at the vertex was averaged separately for each of the 3 stimuli in 500-trial blocks. A "P300" was scored as present if the rare loud click response was significantly more positive in the 200-500 msec range than the frequent response. Paired t-tests of data points across the 40-1000 msec latency period showed that a "P300" was present in every cat before the lesion. The medial septal nuclei and nuclei of the diagonal band were lesioned bilaterally in 10 cats by means of a radio frequency lesion generator and then 12 more recording sessions were carried out before sacrifice and perfusion of the brain. Histological sections from the septum through the hippocampus were stained for AChE and with thionine, for lesion localization. Complete bilateral lesions of the septum resulted in dynamic changes of the "P300". For the first several days the "P300" was normal or enhanced, and , in all cases it became markedly enhanced within the first week and remained enhanced for several days. It then disappeared or decreased below its pre-lesion level. In these animals, with histologically confirmed lesions of the rostral septum and nuclei of the diagonal band, a complete bilateral depletion of AChE in the hippocampus was demonstrated. Incomplete lesion of the septal area produced varying amounts of AChE depletion and varying effects on the "P300", but never complete AChE depletion or marked enhancement of the"P300." These changes in the cat "P300" lead us to conclude that the septal nuclei and nuclei of the diagonal band may influence generation of the P300 through their cholinergic innervation of the hippocampus. (Supported by USPHS AG04088, HD05958 and NS10928).

359.3 CAT "P300": EFFECTS OF HIPPOCAMPAL LESIONS. K. Kaga,* J. Buchwald and J. Harrison. Brain Res. Inst., Ment. Ret. Res. Ctr., Dept. Physiol, UCLA Med. Ctr., Los Angeles, CA 90024.

In previous studies we have reported on a cat model of the human endogenous P300 potential. The cat "P300" is task related, appears in response to rare or omitted stimuli, shows a maximum positivity in the 200-500 msec latency range which, with principal components analysis, shows significantly different factor scores for rare versus frequent stimuli, all characteristics of the human P300. Moreover, in old cats (more than 10 years old) the cat "P300" decreases in amplitude and increases in latency, characteristics of the P300 in older human subjects. In an initial report of possible brain substrates contributing to the cat "P300", primary auditory cortex was bilaterally aspirated in a group of 4 cats. No significant effect was produced by the surgery when 12 sessions of pre-operative "P300" recordings were compared with 12 sessions of post-operative recordings, and we concluded that the cat "P300" was independent of primary auditory cortex. The present study was designed to test the hypothesis that the hippocampus is significantly involved in P300 generation. A group of 5 cats was recorded over 12 sessions pre-operatively. In each case, our typical P300 protocol was presented on a schedule of rare (15%), frequent (80%) and target (5%), and data were collected in 500 trial blocks. Four 500 trial blocks were obtained per recording session, in 2 of which the rare stimulus was a loud click and the frequent stimulus was a soft click, while on the other 2 blocks, these stimuli were reversed. In all cases, the target stimulus was a tone paired with a brief shock to the eyelid to produce a conditioned eyeblink response and focus the animal's attention. After the 12 pre-operative recording sessions were completed, the cat was anesthetized under pentobarbital and surgery was performed under aseptic conditions. Using an approach through the posterior suprasylvian cortex, a small amount of cortical and subcortical tissue was aspirated until the hippocampus could be directly visualized. The hippocampus was then aspirated both dorsally and ventrally. This procedure was carried out bilaterally. Intensive post-operative care was provided as needed, usually for no longer than 24-48 hours. Post-operative recordings commenced 48 hours after surgery and were continued on a daily basis for at least 12 sessions. The animals were then terminated with pentobarbital and the brains perfused. While data analysis is still incomplete, 4 of 5 cats showed a dynamic enhancement of the P300 postoperatively. Two of these 4 have thusfar been processed histologically and, in both, the posterior 30% of the hippocampus had been surgically aspirated bilaterally. These data indicate that partial ablation of the hippocampus results in an enhanced cat "P300" response, and thereby suggest that the hippocampus is involved in P300 generation. (Supported by USPHS grants AG04088, HD05958 and HD04612).

359.4 AN INVESTIGATION OF POSSIBLE PHYSIOLOGICAL ROLES OF THE INTERPEDUNCULAR NUCLEUS. J.R. Leu, T.J. Lynch*, L.E. Jarrard, R.A. Bauman* and J.L. Meyerhoff. Department of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, DC 20307-5100.

Thyrotropin releasing hormone (TRH) has been shown to prevent pentobarbital-induced hypothermia in rats (Kalivas and Horita, Reg. Peptides, 1:335, 1981) by a mechanism apparently separate from its pituitary-thyroid action. Since the interpeduncular nucleus (IPN) is an injection site highly potent for producing this anti-hypothermic effect of TRH, we examined the effect of lesioning the IPN on core temperature responses to pentobarbital (PB) or ambient temperature challenge.

The IPN was radio-frequency (RF) lesioned in 10 rats, while in a group of 10 sham operated controls the RF electrode was lowered to the most dorsal aspect of the IPN and withdrawn. After two weeks recovery, the lesioned and sham rats plus 10 unoperated controls were exposed in separate sessions to 1 hr at 38°C, or 2 hr at 7°C. In a third experiment rats were injected with PB (35mg/kg, i.p.) 2 min following pre-treatment via i.p. injection with either saline or TRH (10mg/kg).

No differences were noted between the 3 surgical groups in baseline temperature or in response to challenge of exposure to hot or cold ambient temperatures. All 3 groups showed the same average PB-induced temperature decrease of 2°C when pre-injected with saline. Pre-treatment with TRH 2 min prior to PB significantly attenuated this hypothermia in all groups. Pretreatment with TRH attenuated PB-induced hypothermia by 93% in the controls, compared to 54% in the lesioned rats, but this difference between surgical groups was not statistically significant.

Total duration of PB-induced sleep was 46% greater in IPN-lesioned rats compared to controls ($p < 0.05$, Tukey's test). Although TRH tended to shorten sleep duration, this effect was not statistically significant in any group. The increase in PB sleep duration in IPN lesioned rats has not been previously reported. Further studies are planned to examine possible homeostatic roles of the IPN.

359.5 THE EFFECT OF FORNIX TRANSECTION ON RESPONSE TO A DISTRACTOR STIMULUS PRESENTED DURING THE FEEDING BEHAVIOR OF RATS. M. J. Epley* and B. Osborne (SPON: T. Root). Department of Psychology, Middlebury College, Middlebury, VT 05753.

The purpose of the present study was to explore the effects of distractor stimulus presentation on the feeding behavior of control and fornix transected Long Evans hooded rats. The rats were given six days of closed economy operant feeding on a continuous reinforcement schedule. The feeding behavior occurred in an environment designed to minimize sensory distraction. During the six days, both feeding and nonfeeding behaviors were recorded continuously. On the last three days a visual distractor stimulus was introduced during meals on a variable ratio three (VR-3) schedule. The study determined that rats with fornix transection did not differ from control rats in overall food consumption. However, fornix transection did result in an increased number of breaks per meal, a decreased intermeal interval, longer meals, more meal drinks and less intermeal drinks. These results were similar to those found in previous studies. Furthermore, stimulus presentation had a differential effect on control and fornix transected rats on measures of breaks per meal, meal size and pellets per meal. The results of the present experiment were interpreted as opposing neither a sensory filtering function of the hippocampus nor a behavioral sequencing function of the hippocampus in terms of feeding behavior. Rather, the hippocampus may be involved in both behavioral sequencing and sensory filtering. A subordinate influence or sensory filtering upon a primary behavioral sequencing function of the hippocampus was suggested.

359.6 SEXUAL DIMORPHISM IN THE EXPLORATORY BEHAVIOR OF RATS WITH FORNIX TRANSECTION. B. Osborne and David Dove*. Department of Psychology, Middlebury College, Middlebury, VT 05753.

The present experiment examined the behavior of male and female Long-Evans rats and sham-operated male and female control rats during the exploration of a novel environment. The rats were given a single 10 min. exposure to a novel environment equipped with environmental supports for exploratory interactions. The behavior was videotaped and analyzed for latency to initiate movement, activity levels, exploration of objects and sequential organization of behavior. Fornix transection resulted in significant changes in all measures. Rats with fornix transection in comparison to controls exhibited increased latency to initiate activity, increased frequency, but decreased duration of both activity and exploration and changes in sequential organization of behavior to a more random pattern. In comparison to males, females also exhibited patterns of exploratory behavior that differed in terms of frequency, duration and sequential organization. The differences between males and females was in the same direction as the difference between controls and fornix transected rats. Fornix transection affected both male and female exploratory behaviors but the effect was greater for males than for females. The results suggest that fornix transection does not result in hyperactivity and hypoexploration. The differences between controls and rats with fornix transection during exploration are more subtle, differing in the frequency, quality, duration and sequencing of behavior. The hippocampus may also be sexually dimorphic in function during exploratin of a novel environment.

359.7 ACTIONS OF ACETYLCHOLINE AND NICOTINE ON NEURONS IN THE RAT SUPRAOPTIC AND PARAVENTRICULAR NUCLEI. J.H. Robinson, S.A. Stwertka, E.P. Christian, V. K. Gribkoff, F.E. Dudek and S.A. Deadwyler. Dept. of Physiology, Bowman Gray Medical School and the R.J. Reynolds Tobacco Co., Winston-Salem, NC, 27103 and Dept. of Physiology, Tulane Univ. Med. Sch., Tulane University, New Orleans, LA 70112.

The supraoptic (SON) and paraventricular (PVN) nuclei of the rat hypothalamus contain magnocellular neuroendocrine cells that synthesize and release vasopressin and oxytocin. Application of acetylcholine (ACh) in the vicinity of the SON promotes vasopressin release from the neurohypophysis by a process involving nicotinic cholinergic receptors (Sladek and Joynt, Endocrinology. 105,367-371, 1979). Electrophysiological evidence also indicates that nicotinic cholinergic activation of SON neurons promotes phasic bursting (Hatton et al., J. Physiol. 345, 297-317, 1983), a firing pattern associated with enhanced hormone release by vasopressinergic neurons. The present study examined the nicotinic cholinergic activation of SON and PVN neurons in the hypothalamic slice preparation.

Nicotine (N) and ACh applied near extracellularly recorded PVN and SON neurons by microdrop (300uM-10mM) or bath delivery (100 - 200uM) increased the firing rates of some of these cells. Application of ACh or N occasionally resulted in phasic bursting in previously silent cells. Pressure application of N from the recording electrode produced systematic increases in the observed excitations with increasing ejection durations and pressures, eventually resulting in depolarization blockade. Addition of the nicotinic cholinergic antagonist d-tubocurarine (d-TC) to the bathing medium blocked or reduced the nicotine excitation in some cells but was ineffective in others. Glutamate elicited excitations were not affected by d-TC. Other neurons were unaffected or depressed by both N and ACh application. Preliminary intracellular recordings suggest that the excitatory effect of nicotine is voltage dependent and is accompanied by a decrease in membrane conductance.

Our data further support the hypothesis that a nicotinic cholinergic mechanism is important in the regulation of neuronal excitability in these hypothalamic nuclei. Although the excitatory actions of ACh and N can promote phasic firing, our studies show that these substances can have complex excitatory and inhibitory effects on PVN and SON neurons.

359.8 INFLUENCE OF ANTEROVENTRAL THIRD VENTRICLE (AV3V) STIMULATION ON THE EXCITABILITY OF HYPOTHALAMIC SUPRAOPTIC NEUROSECRETORY NEURONS. J.H. Jhamandas* and L.P. Renaud (SPON: A.T. Tan). Neurosciences Unit, Montreal General Hospital and McGill University, Montreal, Canada H3G 1A4.

Neurons in the AV3V region can be shown by anatomical and electrophysiological methods to send axons to the supraoptic nucleus, a source of vasopressinergic and oxytocinergic fibers innervating the neurohypophysis. Although the AV3V region is known to participate in the regulation of body fluid balance, the influence of structures in this area on the excitability of supraoptic neurosecretory neurons and thus the release of vasopressin and oxytocin remains uncertain. In the present study we examined supraoptic neurons for their response to electrical stimulation in the AV3V region.

Studies were conducted on pentobarbital anesthetized male Sprague-Dawley rats, using a transpharyngeal route to expose the ventral brain surface for extracellular recordings from supraoptic neurons. Neurosecretory cells were identified by antidromic activation from the neurohypophysis. These were further classified as putative vasopressin-secreting if they displayed phasic or continuous activity and a reduction in firing during peripheral baroreceptor activation induced by a transient rise in blood pressure consequent to intravenously administered metaraminol; cells with continuous activity that lacked blood pressure sensitivity were deemed to be oxytocin-secreting neurons. Monopolar cathodal stimulation (50 usec pulses, 50-150 uA) of the AV3V region was applied through glass insulated tungsten electrodes (tip exposure 50-100 um).

Post-stimulus histogram data obtained from more than 50 neurosecretory neurons revealed that AV3V stimulation could evoke a short latency (4-10 msec) reduction in excitability lasting up to 100 msec from a majority (75%) of putative vasopressin-secreting neurons. In contrast, fewer than 10% of putative oxytocin-secreting cells were responsive; some showed a low probability long duration increase in excitability.

These observations suggest that fibers arising in or passing through the AV3V region exert a selective inhibitory influence on supraoptic vasopressin-secreting neurosecretory cells and are likely to subserve a prominent role in regulating the secretion of antidiuretic hormone from the neurohypophysis (supported by MRC).

359.9 AFTERDISCHARGE IN RAT SUPRAOPTIC NEURONS FOLLOWING REPETITIVE ORTHODROMIC STIMULATION: ROLE OF SYNAPTIC AND INTRINSIC FACTORS. V.K. Gribkoff and F.E. Dudek. Dept. of Physiology, Tulane Univ. Sch. of Med., 1430 Tulane Ave., New Orleans, LA 70112.

Evidence for a projection from cholinergic neurons to magnocellular neuroendocrine cells in the rat supraoptic nucleus (SON) has previously been obtained from anatomical and in vitro electrophysiological studies (Hatton et al., J. Physiol., 345: 297-317, 1983). Single electrical stimuli, applied in the vicinity of these cholinergic neurons dorsolateral to the SON, produce EPSP's in phasically-firing (putative vasopressinergic) SON neurons; stimulus trains could produce an afterdischarge (AD). Dorsolateral stimulation either inhibited or did not affect non-phasic SON neurons. These data suggest that this pathway is important in the regulation of phasic bursting by vasopressinergic neurons. We have further examined the effects of dorsolateral stimulation on SON neurons to delineate the causal factors in the production of AD's.

Intracellular recordings were obtained from SON neurons ($R_i$>100 Mohm, AP>70 mV) of rat hypothalamic slices. We found that single electrical stimuli dorsolateral to the SON produced a short-latency EPSP in all phasic and some non-phasic neurons; repetitive stimulation produced an AD and/or promoted phasic firing in these cells. Stimulation evoked IPSP's in some non-phasic neurons.

Repetitive stimulation produced three effects that could contribute to generation of AD's in those neurons synaptically excited by dorsolateral stimulation. As previously reported by Hatton and coworkers, when neurons were hyperpolarized by intracellular current injection or were not spontaneously active, we consistently observed an increase in frequency of spontaneous PSP's that lasted between 30 sec and several minutes after train cessation. In addition, a slow depolarization followed the stimulus train in several neurons. In some of these neurons the slow depolarization probably resulted from an intrinsic mechanism, because a similar depolarization followed action potentials from intracellular depolarizing current pulses. In other SON neurons, which included phasic cells, a slow depolarization and a decrease in membrane conductance followed stimulus trains. This depolarization could occur in the absence of action potentials during the stimulus train, indicating that this event was possibly synaptic and not due to summation of depolarizing spike afterpotentials.

We have confirmed that repetitive activation of dorsolateral afferents can synaptically activate rat SON neurons, and can promote afterdischarges. Although an increase in spontaneous PSP frequency occurs after dorsolateral stimulus trains, slow depolarizations also appear to contribute to AD. Some of the slow depolarizations apparently arise from intrinsic spike afterpotentials and others may be slow PSP's.

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359.10 **THE EFFECTS OF SYNAPTIC BLOCKADE ON TEMPERATURE-SENSITIVE NEURONS THROUGHOUT THE ENTIRE HYPOTHALAMUS.** J.B. Dean* and J.A. Boulant. Department of Physiology, The Ohio State University, Columbus, Ohio 43210.

Certain hypothalamic regions, such as the preoptic anterior hypothalamus (PO/AH), are known to serve in thermoregulation. Previous single unit studies have used coronal tissue slices and intact animals to study the thermoreceptive properties of PO/AH neurons. The present study used horizontal tissue slices to localize thermosensitive neurons throughout the entire hypothalamus of the rat. These horizontal slices were placed directly over 3 thermodes, which allowed independent control of temperature in the rostral, middle, and caudal regions of each slice. The effects of synaptic blockade on thermosensitive and insensitive neurons were determined by perfusion with a high $Mg^{++}$/low $Ca^{++}$ medium (11.4 mM/0.2 mM). Most of the spontaneously firing neurons were located within 1 mm of the midline and had normothermic firing rates ($FR37^{o}$) of less than 10 impulses/sec. Temperature-insensitive neurons represented the largest population. Warm-sensitive neurons were found throughout the entire hypothalamus with concentrations in the PO/AH, septum. VMH, posterior hypothalamus (PH), and mammillary body (MB). Concentrations of cold-sensitive neurons were found in the PO, PH, and MB. Warm-sensitive neurons outnumbered cold-sensitive neurons in all locations; ranging from 4:1 in the PO/AH to 9:1 in the VMH. Approximately 30% of the warm-sensitive neurons showed hysteresis during local thermal stimulation. While most warm-sensitive neurons displayed a reduced $FR37^{o}$ during synaptic blockade, other warm-sensitive neurons were unaffected. Also during blockade, warm-sensitivity either decreased slightly or remained unchanged. Temperature-insensitive neurons were primarily unaffected by synaptic blockade, but in some cases demonstrated a reduced $FR37^{o}$. This study suggests that while warm-sensitivity is inherent to many hypothalamic neurons, the activity of these neurons is influenced by local synaptic networks, as evidenced by the reduced $FR37^{o}$ and thermosensitivity during synaptic blockade. In addition, the wide distribution of thermosensitive neurons suggests that temperature can affect the hypothalamic control of a variety of regulatory systems. (Supported by NIH & Am. Heart Assoc. grants.)

359.11 A NEW, ROTATIONAL MAZE REQUIRING USE OF VESTIBULAR CUES FOR SPATIAL ORIENTATION: EFFECT OF HIPPOCAMPAL DISCONNECTION. B.L. Matthews*, W.H. Doares*, K.A. Campbell and S.A. Deadwyler. Section on Otolaryngology and Department of Physiology and Pharmacology, Bowman Gray School of Medicine, Winston-Salem, N.C. 27103.

In 1966 Douglas (J. Comp. Physiol. Psychol., 1966) proposed that spontaneous alternation in rats was susceptible to lesions of the hippocampus and that such behavior depended upon vestibular cues for its maintenance. Since that time there has been very little further investigation of the role of vestibular sensory cues in the maintenance of spatial discrimination behavior. In this study we describe a unique form of spatial learning that requires utilization of vestibular sensory inputs and that is sensitive to disconnection of the hippocampus.

A new type of maze was constructed with a short, plus-shaped alley resting on a rotating turntable in the center of an orthogonal 4-arm elevated cross maze. The central, rotating cross was completely covered and consisted of a start box and three separate alleys with curtains blocking the ends to restrict vision. The central cross could be rotated through 360 degrees so that the start box could be halted at one of four possible positions relative to the goal arm which contained a drop of water in a petri dish. Rats were required to use vestibular cues to determine spatial location of the goal on the three choice arms on any given trial. Animals were rotated in the covered cross for 1 to 10 revolutions preceding the 3-choice opportunity, with degree of rotation varying in random sequence across trials.

There were no significant differences in the performance of normal rats (n=6) and fornix-transected rats (n=9) over the first 5 days of training ($M_n$ = 52% correct; $M_{fx}$ = 45.9%, p>.1). However, normal rats improved considerably over the remaining 15-day training period, whereas fornix-cut rats did not: days 16-20 -- $M_n$ = 79.5%, $M_{fx}$ = 51.3%, p<.01). On probe trials with the cover removed, fornix-cut rats were able to use visual cues, and performed the task as effectively as normals, indicating that the impairment in fornix-cut rats was due to a specific deficit in the vestibular spatial task. When tested for ability to perform correctly on 90 degree rotations from the goal arm, fornix cut animals were deficient (20% below) with respect to normals at the beginning of each daily session but improved over the 20 day testing period such that at the end of the daily session this deficit was eliminated. The results confirm the original supposition by Douglas with regard to the role of the hippocampus as critical for processing vestibular sensory information for spatial orientation.

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360.1 REGIONAL VARIATIONS IN THE CHOLINERGIC INNERVATION OF THE PRIMATE CORTICAL SURFACE. M. Mesulam, L. Volicer, J. Marquis, E. Mufson, R. Green. Harvard University and Boston University, Boston, MA

The enzyme choline acetyltransferase, a specific marker for the presynaptic component of cholinergic pathways, was measured in 33 cytoarchitectonic subregions of the Macaque cortex. As expected, the hippocampus and amygdala contained high levels of this marker. In the other parts of the cortical mantle, up to seven-fold differences were noted from one region to another. For example, the paralimbic (mesocortical) regions of the brain (e.g. parahippocampal, insular, caudal orbitofrontal and temporopolar areas) contained high levels of choline acetyltransferase. In contrast, the concentration of this cholinergic marker was the lowest within all frontal and temporo-parietal association areas. As a group, the primary sensory-motor regions contained an intermediate level of this enzyme. Choline acetyltransferase activity also showed a gradual increase from the isocortical towards the more primitive periallocortical subsectors of the paralimbic areas. Each of these differences was statistically significant ($p<.05$). The one exception to this plan of organization was presented by the cingulate cortex which - despite being a paralimbic region -contained among the lowest levels of choline acetyltransferase. Regional acetylcholinesterase activities were also assayed and showed variations which closely reflected those of choline acetyltransferase ($p<.01$, $r = .9$).

The selective concentration of cholinergic terminals within paralimbic and limbic areas may help to explain the relatively specific effects of systemically administered cholinoactive agents upon mood and memory. Although a depletion of cortical cholinergic input is an important component of Alzheimer's disease, it is interesting that the regional variations of cholinergic innervation do not help to predict the neuropathological predilection patterns that are known to exist in this condition. For example, prefrontal and temporoparietal association areas which are highly susceptible to plaque formation in Alzheimer's disease appear to have extremely low levels of natural cholinergic innervation.

This regional distribution also suggests that cortical cholinergic innervation is likely to play a pivotal role in the transmission of sensory information into the limbic system. For example, paralimbic areas which are very important in sensory limbic interactions have a high cholinergic innervation. Furthermore, in each of the three major sensory modalities, we found that the part of modality-specific association cortex which sends the most projections into the amygdala contains the highest level of cholinergic innervation. This anatomical distribution pattern suggests that cholinergic pathways may play their well known role in memory by gating motivationally relevant neural templates into and out of memory storage. Supported in part by the McKnight Foundation, the ADRDA Faculty Scholar Award, NS20285, AG05134, DOD-ARO-DAAG-79-K-820042 and the VA.

360.2 EXTRACELLULAR RECORDING FROM NUC. DIAGONAL BAND AND OTHER BASAL FOREBRAIN NEURONS IN THE RAT BRAIN SLICE. C. P. VanderMaelen, G. Gehlbach, R. C. Wilderman*, and A. S. Eison, Preclin. CNS Res., Bristol-Myers Co., Evansville, IN 47721.

Cholinergic neurons of the basal forebrain are implicated in senile dementia of the Alzheimer type. Previous extracellular recordings in vivo (Aston-Jones et al., 1983, 1984) and intracellular recordings in cell culture (Nakajima et al., 1984) from basal forebrain neurons have been made. The present study established a brain slice preparation for recording from cholinergic and non-cholinergic neurons of the rat basal forebrain. Chloral hydrate anesthetized male albino Sprague-Dawley rats, 100-150 g., were allowed to breathe 100% $O_2$ for at least 5 min. Frontal slices 400 µm in thickness were cut through the basal forebrain at the level of the anterior commissure using a Sorvall TC2 Tissue Sectioner. In 2 rats parasaggital sections were cut. Slices were incubated in a chamber containing 95% $O_2$, 5% $CO_2$ and bathed in continuously flowing artificial CSF of the following composition in mM: NaCl, 130; KCl, 5.0; $CaCl_2$, 1.25; $MgSO_4$, 1.25; $NaHCO_3$, 24.0; $NaH_2PO_4$, 1.25; D-glucose, 10.0. Extracellular single-unit recordings, usually aimed at the horizontal limb of the nucleus of the diagonal band of Broca (HDB), were made using glass micropipettes filled with 2M NaCl and 0.5% Pontamine Sky Blue. At the end of an experiment a dye spot was deposited in the slice, which was fixed in 10% formalin with 25% sucrose. 40 µm sections were cut, stained with neutral red, and locations of recorded neurons reconstructed.

Extracellular single-unit recordings were made from 98 neurons in 41 experiments. Neurons varied widely in action potential shape and spontaneous discharge rate. Action potentials were monophasic (positive or negative), biphasic (pos.-neg.) or triphasic (pos.-neg.-pos.), and were usually 0.7-2.0 msec in duration. Spontaneous discharge rates varied from 0 to 37 Hz. For 60 cells the muscarinic agonist carbachol was administered (0.1-100 µM). The vast majority (81.7%) of these cells were excited by carbachol, while 13.3% were inhibited, and 5.0% showed no response. Type of response to carbachol could not be predicted on the basis of spike shape or spontaneous discharge rate. In 77% of the experiments in which it was tried (N=13) the muscarinic antagonists atropine (0.5-100 µM) or scopolamine (0.5-1.0 µM) totally or greatly blocked the responses induced by carbachol. Histological verifications showed that most recorded neurons were located in the HDB or adjacent basal forebrain areas including substantia innominata, magnocellular preoptic nucleus, and vertical limb of the nucleus of the diagonal band. Some dye spots were located near clusters of magnocellular neurons suggesting that some recordings may have been from cholinergic neurons. Further studies will be required to determine if the cholinergic neurons can be identified by electrophysiological or pharmacological criteria.

360.3 BASAL FOREBRAIN NEURONS PROJECTING TO THE RAT CEREBRAL CORTEX: A MICROIONTOPHORETIC STUDY. P. Dutar*, O. Rascol*, A. Jobert* and Y. Lamour, INSERM U. 161, 2 rue d'Alésia, 75014 Paris (France).

The mammalian cerebral neocortex receives a diffuse cholinergic innervation from neurons located in the basal forebrain. Many of these neurons are located in the substantia innominata (SI) and in the ventral globus pallidus (GP). We recorded such basalo cortical neurons (BCNs) located in the ventro medial GP (nucleus basalis) and SI, and antidromically driven by the electrical stimulation of the frontal and parietal cortex in urethane anaesthetized rats. Conventional amplification methods were used to record neuronal activity from micropipettes filled with 1 M NaCl and 2% pontamine sky blue. These pipettes were rigidly attached to multibarreled micropipettes filled with solutions for testing by iontophoresis. The position of the BCNs was marked with a dye deposit, and the electrodes tracks subsequently reconstructed on coronal brain sections.

Sixty one BCNs were recorded from twenty eight animals. The location of the BCNs was in good agreement with previous anatomical data. BCNs were antidromically driven with latencies of 1.1 to 13.5 msec, giving conduction velocities of 0.6 to 6.8 m/s. (mean: 3.1 m/s). A majority of them was driven by the stimulation of the frontal electrodes. In some cases, evidence suggestive of axonal branching was obtained. Many BCNs had a very regular pattern of spontaneous discharge (mean spontaneous activity: 20 impulses per second). Most of the BCNs were not responsive to non-noxious peripheral somatic stimulation. BCNs were readily excited by the iontophoretic application of glutamate and strongly inhibited by GABA. Eighty-five percent of the BCNs could be excited by acetylcholine. No inhibition was observed. They could also be excited by cholinergic agonists. Muscarinic agonists excited a higher proportion of BCNs than nicotinic agonists. The most effective agonists were (ranked in order of decreasing efficacy): carbamyl methylcholine, acetyl methylcholine, muscarine, oxotremorine, carbachol, butyrylcholine, choline, TMA, DMPP. Excitatory responses to acetylcholine, carbachol and muscarinic agonists were abolished by atropine but rarely by nicotinic antagonists. It is known, on anatomical grounds, that most BCNs located on SI and GP are cholinergic neurons. Our results show that these central cholinergic neurons have relatively high spontaneous discharge rates and axonal conduction velocities. They also show that they are not inhibited but rather excited by their own transmitter, as well as by several cholinergic agonists.

360.4 CORTICALLY PROJECTING MAGNOCELLULAR BASAL FOREBRAIN NEURONS IN THE RAT. I. PHYSIOLOGY. P.B. Reiner, K. Semba, H.C. Fibiger and E.G. McGeer. Div. of Neurological Sciences, Dept. of Psychiatry, Univ. of British Columbia, Vancouver, B.C. V6T 1W5 Canada.

The magnocellular neurons of the basal forebrain provide a major extrinsic cholinergic input to the cerebral cortex. As a first step towards elucidating the functional properties of this cholinergic system, we have studied the physiological characteristics of cortically projecting magnocellular basal forebrain (CP-MBF) neurons using extra- and intracellular recordings in conjunction with morphological analyses employing intracellular HRP staining (see companion abstract).

In chloral hydrate anesthetized rats, 19 neurons satisfied criteria of constant latency and high frequency following to antidromic stimulation of cingulate (N=10) or frontal (N=9) cortices; in 4 spontaneously active neurons, collision was demonstrated as well. A sub-group of 6 CP-MBF neurons exhibited frequent loss of the somato-dendritic (SD) portion of the antidromically generated action potential. This phenomenon was clearly dependent upon stimulation frequency: in one neuron studied in detail, the initial segment spike followed faithfully at 100 Hz, while the SD segment disappeared as stimulation rates were increased from 1 to 2 Hz. SD failure was never seen during spontaneous action potentials. In contrast, the remaining CP-MBF neurons (N=13) showed no change in spike shape even at stimulus frequencies greater than 250 Hz. CP-MBF neurons exhibiting SD failures also had longer latencies to antidromic invasion (16.8 ± 6.2 vs. 3.0 ± 2.5 msec, mean ± SD, $p<0.001$) than their non-failure counterparts.

While these data demonstrate that physiological distinctions among CP-MBF neurons exist, similarities were noted as well. Although all CP-MBF neurons displaying the SD failure phenomenon had little or no spontaneous activity in these anesthetized rats, this did not serve as a distinguishing feature as 10/13 non-failure neurons were also relatively silent. Furthermore, neither the anatomical locations nor the cortical terminations (as evidenced by cortical stimulation site) of these sub-groups of CP-MBF neurons were distinct. Finally, both populations consisted of magnocellular neurons, although some morphological differences could be appreciated (see companion abstract).

In conclusion, we have identified a sub-group of CP-MBF neurons with unique and easily identifiable physiological characteristics, namely SD failure and long latency to antidromic activation. Although the majority of CP-MBF neurons can be expected to be cholinergic, the physiological heterogeneity displayed by these neurons calls for definitive characterization of the transmitter status of physiologically identified neurons.

(Supported by the Medical Research Council of Canada)

360.5 CORTICALLY PROJECTING MAGNOCELLULAR BASAL FOREBRAIN NEURONS IN THE RAT. II. MORPHOLOGY. K. Semba, P.B. Reiner, E.G. McGeer and H.C. Fibiger. Div. of Neurological Sciences, Dept. of Psychiatry, Univ. of British Columbia, Vancouver, B.C. V6T 1W5 Canada.

In recent years, anatomical studies have revealed the existence of a topographically organized projection from the magnocellular basal forebrain to the neocortex. Several studies have shown that a majority of cortically projecting magnocellular basal forebrain (CP-MBF) neurons are cholinergic. To examine in detail the morphology of CP-MBF neurons, we injected horseradish peroxidase (HRP) intracellularly into antidromically identified cells following physiological characterization (see companion abstract).

Three CP-MBF neurons which were sufficiently well stained with HRP were reconstructed for morphological examination at the light microscopic level. The 3 cells were located, respectively, in the magnocellular preoptic area (MPA), the nucleus of the horizontal limb of the diagonal band (HLDB), and the globus pallidus-internal capsule (GP-IC) border. Physiologically, all 3 cells were driven from the cingulate cortex, and the cell in the MPA displayed the phenomenon of somato-dendritic spike failure, whereas the other two cells did not (see companion abstract).

Morphologically, all 3 CP-MBF cells had large somata (longest dimensions over 40 μm), and were isodendritic. Dendrites could be followed over 600 μm from the soma; some dendrites appeared beaded. In the MPA and GP-IC cells, the presumptive axons arose from either the soma or proximal dendrite and appeared to give off local collaterals (the axon of the HLDB cell could not be identified). Although the 3 CP-MBF cells thus showed some general similarities in morphology, some differences were noted as well. For example, the dendrites of the MPA cell ramified more frequently than did those of the other two cells. The pattern of dendritic arborization in the MPA and HLDB cells was radially oriented, while the cell located at the GP-IC border displayed a pattern extremely skewed towards the core of the GP. In addition, the dendrites of HLDB and GP-IC cells were moderately spiny distally, but relatively aspiny proximally, whereas those of the MPA cell were moderately spiny throughout.

The above observations indicate that CP-MBF cells in the rat display similar morphological features including large cell size, isodendriticity, and presence of spines. However, differences in terms of dendritic arborization patterns and distribution of spines were noted as well, which may correlate with differences in physiological characteristics. Current efforts are directed towards determining if these CP-MBF neurons are indeed cholinergic.

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360.6 Cholinergic Projections from the Parabigeminal Nucleus (Ch8) to the Superior Colliculus in the Mouse. E.J. Mufson, T.L. Martin, D.C. Mash, B.H. Wainer and M-M. Mesulam, Harvard Med. Sch., Boston, MA. and University of Chicago, Chicago, IL.

Seven cholinergic cell groups extending from the basal forebrain to the mesencephalic tegmentum have been designated Ch1-Ch7 (Mesulam et al '83). The Ch1-Ch4 sectors are located within the basal forebrain and provide the primary cholinergic innervation for the telencephalon; the Ch5-Ch6 sectors are in the pontomesencephalic region and provide cholinergic projections to the thalamus; the Ch7 sector is in the habenula and projects to the interpeduncular nucleus. This study demonstrates an additional brainstem cholinergic cell group located within the parabigeminal nucleus which provides a major cholinergic projection to the superior colliculus (SC). The cholinergic cells located within this nuclear region are designated Ch8.

Peroxidase anti-peroxidase immunocytochemical staining with monoclonal antibodies to choline acetyltransferase (ChAT) in male CD-1 mice revealed intensely ChAT positive oval cell bodies (10-20 um in diameter) within the parabigeminal nucleus. ChAT processed tissue counterstained with thionin showed that approximately 80-90% of the perikarya of this region are cholinergic. Animals were also prepared for muscarine receptor autoradiography. Autoradiographic visualization of tritiated QNB binding with sufficient pirenzipine to mask $M_1$ sites revealed intense $M_2$ receptor labeling over the region corresponding to Ch8.

We injected small quantities of HRP-WGA into the SC. Retrogradely labeled neurons were observed in the contralateral and ipsilateral parabigeminal nuclei (Ch8). In tissue processed for the concurrent demonstration of ChAT and HRP, many HRP labeled neurons in the parabigeminal nucleus (Ch8) were also ChAT positive. The number of double labeled neurons was four times greater in the contralateral as compared to the ipsilateral parabigeminal nucleus (Ch8). Although no double labeled neurons were found in Ch1-Ch4 and Ch7, a few ChAT-HRP positive cells were seen in Ch5-Ch6, bilaterally. In contrast, an HRP injection into the inferior colliculus resulted in the co-localization of ChAT and HRP only in Ch5, ipsilaterally.

Tissue processed for ChAT and AChE histochemistry as well as for muscarine receptors revealed intense bands of these cholinergic markers in the superficial and deep layers of the SC. This shows that the SC receives a substantial cholinergic input and it appears that the parabigeminal nucleus (Ch8) is a major source of this cholinergic innervation. The demonstration of brainstem cholinergic neurons within Ch5 (pedunculopontine nucleus) and Ch6 (lateral dorsal tegmental nucleus) and now in Ch8 (parabigeminal nucleus) indicates the need for a more complete analysis of these regions in diseases associated with central cholinergic dysfunction. Supported by NS 20285, NS 029211, NS 17661, AG 05134, the ADRDA, and the McKnight Foundation.

360.7 MORPHOLOGIC CHARACTERISTICS OF CENTRAL CHOLINERGIC NEURONS IN THE RAT. L. L. Butcher, E. Gould* and N. J. Woolf, (SPON: R. W. Jacobs). Dept. of Psychology and Brain Research Institute, University of California, Los Angeles, CA 90024.

Morphologic assessments were performed on cholinergic neurons, specifically those that were both immunoreactive for choline acetyltransferase and stained for acetylcholinesterase. Neurons were randomly chosen from all brain regions containing cholinergic cells, and these neurons were assessed for maximal soma extent, minimal soma extent, soma shape, number of processes, degree of branching of processes, and the lengths of processes.

Intrinsically organized cholinergic neurons found in the caudate-putamen complex, nucleus accumbens, and olfactory tubercle had similar average maximal and minimal soma extents (20.9-21.5 μm and 15.0-15.6 μm, respectively). Basal forebrain cholinergic cells had maximal soma extents ranging from 19.5 μm in the lateral preoptic area to 23.4 μm in the nucleus basalis. Cholinergic cells in the pontine tegmentum were slightly larger on average (maximal soma extent in dorsolateral tegmental nucleus: 25.5 μm; pedunculopontine tegmental nucleus: 22.9 μm). In most cranial nerve nuclei the cholinergic somata were the largest found (e.g., average maximal soma extent in the motor nucleus of cranial nerve V: 32.5 μm), but cholinergic neurons found in the nucleus of cranial nerve X were considerably smaller (average maximal extent: 15.3 μm).

In general oval shapes were most common for cholinergic somata; e.g., 80% of the cholinergic cells were oval in both the caudate-putamen and the nucleus of cranial nerve X. Significant proportions of fusiform-shaped cholinergic cells were found prominently in the nucleus basalis, dorsolateral tegmental nucleus, and pedunculopontine tegmental nucleus.

Cholinergic cells in the striatum, basal forebrain, pontine tegmentum, and cranial nerve nuclei III, IV, V, and VII were observed with as many as 5-7 processes having extents up to 35-65 μm. A maximum of 2 processes having extents up to 15 μm were found for cells in the nuclei of cranial nerves X and XII.

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360.8 ATTENUATION OF THE PHOTORESPONSE IN EXTRARETINAL PHOTORECEPTOR CELLS OF APLYSIA CALIFORNICA BY CHOLINESTERASE INHIBITORS. J.P. Apland. Sensory Research Division, U.S. Army Aeromedical Research Laboratory, Fort Rucker, AL 36362-5000.

Aplysia extraretinal photoreceptor (ERP) cells $R_2$, $LPl_1$, and VPN (Andresen and Brown, Experientia 38:1001, 1982) were used as models of phototransduction. The early steps of light transduction in Aplysia ERP cells are very similar to those proposed in the calcium scheme for vertebrate rod outer segments (Hagins, Ann. Rev. Biophys. Bioeng. 1:131, 1972). The effects of cholinesterase inhibitors and their antidotes on photoresponses in Aplysia ERP cells were investigated by electrophysiological methods.

Bath application of diisopropyl fluorophosphate (DFP), a potent irreversible organophosphate-type cholinesterase inhibitor, consistently decreased the maximum amplitude of the photoresponse elicited by flashing a light on Aplysia ERP cells. DFP did not change membrane resistance, nor did it change the reversal potential for the photoresponse. Physostigmine, a reversible carbamate-type cholinesterase inhibitor, depressed both photoresponse amplitude and membrane resistance. Attenuation of photoresponse was dose-dependent with both DFP and physostigmine and was completely reversed by washing out the drugs. Physostigmine was less potent. Both drugs caused depolarization of the resting membrane potential (RMP). Pyridostigmine, another reversible carbamate-type cholinesterase inhibitor, had no effect on photoresponse or membrane resistance at 5 times the concentration used for physostigmine. Bath application of carbachol, which would mimic a buildup of acetylcholine (ACh) following cholinesterase inhibition, caused a persistent hyperpolarization of the RMP. Carbachol caused attenuation of both photoresponse and membrane resistance. Jovic et. al. (Biochem Pharmacol. 20:519, 1971) have shown that DFP inhibits Na, K-ATPase. Therefore, it seemed that the depolarization caused by DFP might be caused by inhibition of the electrogenic Na pump in Aplysia ERP cells. However, superfusion with DFP still caused the usual depolarization after treatment of ERP cells with $10^{-3}$M ouabain, suggesting that the depolarization is not caused by Na pump inhibition. The muscarinic ACh receptor antagonist, atropine, blocked the photoresponse attenuation caused by DFP. Atropine did not block the attenuation of photoresponse and membrane resistance caused by physostigmine and carbachol. Calcium-free, high-magnesium sea water, which blocks release of ACh and other neurotransmitters, did not block the attenuation of photoresponse caused by DFP.

The effects of DFP are reversible, and different from those of physostigmine and carbachol. Calcium-free sea water did not block DFP's effects. These results suggest that the effects of DFP on ERP cells are not due simply to a buildup of ACh at synapses subsequent to cholinesterase inhibition. Atropine's block of DFP's effects might be caused by competition for binding sites.

360.9 EVIDENCE OF CHOLINERGIC INVOLVEMENT IN REPRODUCTIVE MECHANISMS IN NORMAL AND IN NEONATALLY ANDROGENIZED FEMALE RATS. M.V. Wagster* and N.W. Pedigo, Jr. (SPON: A.A. Gerall). Dept. of Psychology, Tulane Univ., New Orleans, LA 70118, and Dept. of Pharmacology, Louisiana State University Medical Center, New Orleans, LA 70112.

Previous research has identified the cholinergic system as a critical component for both behavioral and gonadotropic processes of the female rat. In the present study, changes in 3H-quinuclidinyl benzilate (3H-QNB) binding to CNS muscarinic receptors were compared to changes in ovulatory and behavioral mechanisms in the neonatally androgenized female rat. Sprague-Dawley females were injected with 1 ug testosterone propionate (TP), 1000 ug TP, or oil on Day 5 of life. Animals were ovariectomized at approximately 100 days of age. Injections of 3.3 ug of estradiol benzoate (EB), 72 and 48 hours before testing, and .05 mg of progesterone 2-4 hours before testing, were given and lordosis behavior was observed. Three weeks after the final behavioral test, animals were injected with either 3.3 ug of EB or oil at 72 and 48 hours before sacrifice. The incidence of regular estrous cycles and lordosis behavior decreased as a function of increasing dosage of neonatal testosterone. The ovaries from every control animal contained evidence of corpora lutea while those in 1 ug TP and 1000 ug TP had little or no evidence of corpora lutea at the time of ovariectomy. Exposure to 1000 ug TP significantly inhibited lordosis while 1 ug of TP allowed females to continue to exhibit lordosis behavior at a level only slightly below that of control females. Androgenization of the animals significantly reduced binding of 3H-QNB in the ventromedial hypothalamus (VMH). The mean levels of specific 3H-QNB binding expressed as fmol/mg protein were 800.03 for the controls, 737.17 for the 1 mg TP groups, and 583.08 for the 1000 mg TP groups. Furthermore, administration of EB prior to sacrifice significantly decreased specific 3H-QNB binding in VMH. The mean level of specific 3H-QNB binding for the groups receiving estrogen was 632.80 fmol/mg protein and for the groups receiving oil, 781.25 fmol/mg protein. Binding was not altered significantly in the medial preoptic area, the midbrain central gray, or the corpus striatum as a function of neonatal androgen treatment or estrogen administration. Conflicting results were obtained from this report in comparison to other work on the effect of estrogen on muscarinic receptor binding in the VMH. We propose that differences in the timing of EB administration, amount of EB given, and/or preparation of the tissue for the 3H-QNB assay may have been factors in the differences which were obtained. Furthermore, the decrease in muscarinic receptor binding in VMH and the trend for decreased binding in the mPOA as a result of neonatal androgenization may have important implications for the role of the cholinergic receptor in normal age-related declines in female reproductive capability.

360.10 ENHANCED $^{45}Ca$ UPTAKE AND $^{86}Rb$ RELEASE FROM RAT CEREBRAL CORTEX DURING SIMULATED HYPOGLYCEMIA. J.M.Gorell,B.Czarnecki* and S. Hubbell*.Dept.of Neurology,Henry Ford Hospital,Detroit, MI 48202.

To define some ionic mechanisms underlying reported(Gorell et al Soc.Neurosci.,1984)changes in acetylcholine(ACh) release during time- and (glucose)-graded states of simulated hypoglycemia(HG) in vitro,the uptake of $^{45}Ca$ and release of preloaded $^{86}Rb$ were studied.

In $^{45}Ca$ uptake experiments,cerebral cortex(CX) slices from adult male Sprague-Dawley rats were incubated(37°C,15min) in Krebs-$HCO_3$ (K-b) buffer containing either 0.25,0.1,0.05(HG) or 5(control)mM glucose(glc) with constant gassing(95%$O_2$:5%$CO_2$).Following 20,60 or 80 min HG induction,$^{45}Ca$ uptake(10μCi/tube) was measured during a 10 min incubation in either 3 or 60 mM KCl K-b. In $^{86}Rb$ release studies CX slices preloaded with $^{86}Rb$(60μCi/7ml)during a prior 5 mM glc incubation(37°C,45min)were superfused with K-b containing 0.05,0.1, 0.25 or 5 mM glc in 3 mM KCl for 20 or 60 min,followed by 5 min of 60 mM $K^+$-stimulation.

There was a significant increase in $^{45}Ca$ uptake in 3 mM KCl K-b vs. controls during 0.25 mM glc exposure (20 min: 132±7%, n=6,p<.01; 60 min: 177±7%,n=6,p<.001;80 min: 127±5,n=5,p<.001), with 0.1 mM glc (20 min: 134±7%,n=6,p<.001;60 min: 147±5%,n=6,p<.001;80min: 149±6%, n=5,p<.001), and with 0.05 mM glc (20 min: 149±8%,n=6,p<.001; 80 min: 138±3%,n=6,p<.001). A slight decrease in 60 mM KCl-induced $^{45}Ca$ uptake was found with 0.25 mM glc (20 min: 83±2%,n=5,p<.02;60 min: 68 ±3%,n=6,p<.001) with 0.1 mM glc (20 min: 85±2%,n=4,p<.05;60 min: 70 ±2%,n=6,p<.001;80 min: 89±3%,n=6,p<.05), and with 0.05 mM glc (20 min: 75±3%,n=6,p<.05;80 min: 88±4%,n=6,p<.01). At 20 min of HG induction, there was a slight elevation is basal (3 mM KCl) $^{86}Rb$ efflux with 0.05 mM glc (109±1%,n=12,p<.001) and no change from control with either 0.1 or 0.25 mM glc. At 60 min of HG induction, there was a significant rise in basal (3 mM KCl) $^{86}Rb$ efflux with 0.05 mM glc (130±1%,n=18,p<.001), with 0.1 mM glc (122±1%,n=24,p< .001), and with 0.25 mM glc (114±2%,n=30,p<.001). This $^{86}Rb$ release was glc-dependent (correlation coefficient -0.96). $^{86}Rb$ release during 60 mM KCl stimulation was the same in HG and control CX slices.

These observations of a HG induced increase in basal $^{86}Rb$ efflux and $^{45}Ca$ uptake strongly suggest that a state of sustained partial CX slice depolarization exists in HG, resulting in an increased ($^3H$)-ACh release under normal (3 mM KCl) conditions, as reported previously. During KCl stimulation, $^{86}Rb$ efflux and $^{45}Ca$ uptake appear to play less of a role in regulating ACh release during HG since both processes do not act at restrictive levels.

This research was supported by the Fund for Henry Ford Hospital and by the Gossett Fund.

360.11 ETHOXYCHOLINE MUSTARD: A NON-HYDROLYSABLE DERIVATIVE OF ACETYLCHOLINE MUSTARD. E.H.Colhoun*, R.Gregoire* and R.J.Rylett (SPON. C.J.Gibson). Department of Pharmacology and Department of Physiology, University of Western Ontario, London, Canada.

We have shown that the aziridinium ion species of choline mustard (ChM Az) and monoethylcholine mustard (ECM Az) are useful neurochemical probes for investigations relating to the regulation of acetylcholine (ACh) synthesis. In particular, these nitrogen mustard analogues of choline are potent inhibitors of high-affinity choline transport at cholinergic nerve terminals but weak inhibitors of choline acetyltransferase. When administered to rodents both ChM Az and ECM Az produced a hemicholinium-like toxic reaction characteristic of presynaptic cholinergic blockade. We have synthesized and studied the effects of ethoxycholine (ethylethercholine) mustard aziridinium ion (EOCM Az) which differs in chemical structure from acetylcholine insofar that the carbonyl of the ester linkage has been replaced by a methylene group but like the other choline mustards has the strained three membered ring structure with the potential to alkylate nucleophilic centers in biological macromolecules. It was predicted that this molecule could inhibit AChE but would not be a substrate for the enzyme, would be a poor inhibitor of choline transport and could be a ligand for ACh receptors. EOCM Az proved to be a weak inhibitor of synaptosomal choline uptake with a rank order of potency of the compounds tested to be : ChM Az > ECM Az > AChM Az > EOCM Az

( 1 : 2.3 : 27.8 : 400 )

EOCM Az was an agonist at both muscarinic and nicotinic ACh receptors and relative to carbachol it was found to be 13 and 40 times less potent, respectively. In other experiments, alkylation of muscarinic receptor sites in isolated guinea-pig ileum strips by EOCM Az was not detected, however, in homogenates of the strips, evidence was obtained that EOCM Az bound irreversibly to the receptor sites (using [$^3H$]QNB as the ligand). EOCM Az was also found to irreversibly inhibit AChE with evidence of alkylation of the anionic enzyme site. EOCM Az produced signs of acute cholinergic toxicity in mice, similar to that observed for AChM Az, but unlike ChM Az and ECM Az there was no evidence of hemicholinium-like toxicity. This result correlated with the evidence that EOCM Az did not produce presynaptically-mediated blockade of the twitch response in the isolated rat phrenic nerve-diaphragm preparation. Acute $LD_{50}$ values in mice were found to be 10 mg/kg and 128 mg/kg for i.v. and i.p. routes, respectively: evidence for delayed cytotoxicity was obtained at a dose of 80 mg/kg i.p.

(Supported by MRC Canada and NSERC Canada)

360.12 DEPOLARIZATION ENHANCES INHIBITION OF SYNAPTOSOMAL CHOLINE ACETYLTRANSFERASE BY CHOLINE MUSTARD AZIRIDINIUM ION. R.J.Rylett and E.H.Colhoun*. Department of Physiology and Department of Pharmacology, University of Western Ontario, London, Canada.

Depolarization of synaptosomes with potassium or veratridine enhances the velocity of sodium-dependent choline transport. Although the mechanism(s) regulating the velocity of the carrier have not been elucidated, it would appear that either the cytoplasmic acetylcholine (ACh) concentration or the ACh release process may be involved. Previously, we reported (Soc.Neurosc., 10: 1180) that irreversible inhibition of high-affinity choline carriers by choline mustard aziridinium ion (ChM Az) was increased following depolarization of rat brain synaptosomes. This result would indicate that during repolarization of the nerve terminals a greater number of choline carrier binding sites were exposed at the outer membrane surface per unit time. We now report that, in addition to the enhanced inactivation of high-affinity choline carriers by ChM Az during repolarization, irreversible inhibition of synaptosomal choline acetyltransferase (ChAT) activity was increased. Rat forebrain synaptosomes were incubated in Krebs-Ringer (KR) buffer ($K^+$, 5 mM) or high $K^+$-KR ($K^+$, 40 mM) for 10 min at 37°C then exposed to ChM Az (0.9 μM) for 10 min (in regular KR) before $^3H$-choline transport activity was monitored. $^3H$-Choline uptake velocity was increased by 40% in control synaptosomes but was not enhanced in ChM Az-treated synaptosomes; inhibition of $^3H$-choline transport was increased from 46% to 61% in the previously depolarized nerve terminals. Inhibition of synaptosomal ChAT by ChM Az was increased from 17% to 26% by prior $K^+$-depolarization. The omission of sodium from the external medium abolished the depolarization-induced responses. The inactivation of synaptosomal ChAT cannot be easily explained by simple accumulation of ChM Az within the nerve terminals due to the rapid irreversible blockade of choline carrier sites. The correlation between increased inhibition of choline carriers and ChAT suggest a coupling between these two components regulated by neuronal activity.

(Supported by a grant from MRC Canada)

360.13 CHOLINERGIC NEUROPATHOLOGY FOLLOWING INTRACEREBRAL INFUSION OF ETHYLCHOLINE MUSTARD AZIRIDINIUM (AF64A). Susan R. McGurk and Larry L. Butcher. Department of Psychology and Brain Research Institute, University of California, Los Angeles, CA 90024.

Recent studies have indicated that an alkylating analog of choline, AF64A, creates a selective long-term cholinergic hypofunction. Such an effect would be invaluable in the examination of central cholinergic mechanisms. In an effort to evaluate further cytopathological effects of AF64A on cholinergic neurons, the striata or nuclei basalis of 84 adult rats (Sprague-Dawley strain) were stereotaxically and unilaterally injected with this compound. Doses were 0.02, 0.05, 0.1, 0.2, 0.25, 0.4, 0.5, 1.0, 2.0, 2.5, 5.0, 10.0, or 20.0 nmol of AF64A. The vehicle volumes included 0.5, 1.0, 2.0, 5.0, or 10.0 µl of 0.9% saline adjusted to pH 7.4 with NaOH. Control injections were 1.0, 2.0, 2.5, 5.0, or 10.0 µl of the vehicle. Subjects were sacrificed 2, 7, or 14 days post-treatment. The brains were processed subsequently for acetylcholinesterase, NADH-diaphorase, and Nissl substance as detailed in Butcher and Bilezikjian (1975, Eur. J. Pharmacol. 34, 115-125). Histological examination of both the caudate-putamen complex and nucleus basalis revealed gross non-selective tissue damage resulting from 1-20 nmol of AF64A. For the striatum, doses in the range 0.1-0.5 nmol produced minimal non-specific damage with moderate effects on cholinergic indices, while doses below 0.1 nmol did not produce significant neuronal destruction. In the nucleus basalis, doses of 0.1-0.5 nmol produced unacceptable non-selective damage, with neither non-selective tissue damage nor appreciable neuronal pathology resulting from the smallest dose range of 0.02-0.05 nmol of AF64A.

These data indicate marked differences in the cytotoxic effect of AF64A as a function of region infused. They would further suggest that careful evaluations of the parameters of dose, volume of injections, and rate of infusion, as well as of the histopathologic consequences of AF64A, must be performed before the toxin is used in behavioral and physiological studies.

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360.14 EFFECTS OF ORGANOPHOSPHATE ANTIDOTES ON BRAIN CHOLINESTERASE ACTIVITY AND ACETYLCHOLINE LEVELS. T.-M. Shih, T. Koviak*, O. Smith*, A. Kaminiskis* and V.R. Jimmerson. Basic Pharm. Br., U.S. Army Med. Res. Inst. of Chem. Def., APG, MD 21010-5425

The toxicity of organophosphate cholinesterase (ChE) inhibitors is attributable to the inhibition of ChE activity and the subsequent elevation of acetylcholine (ACh) levels. Antidotes to these ChE inhibitors may alleviate the toxicity by reducing the degree of ChE inhibition and/or ACh elevation. We examined the effects of several antidotes, anticholinergics, anticonvulsants or oximes, to a potent organophosphate ChE inhibitor, soman, on brain ChE activity and ACh levels. Male rats were injected with atropine sulfate (16 mg/kg, i.m.), atropine methylnitrate (17 mg/kg, i.m.), aprophen (100 and 200 mg/kg, i.m.), diazepam (2.5 and 5.0 mg/kg, i.m.), 2-PAM (43 mg/kg, i.m.), or HI-6 (125 mg/kg, i.p.) in the presence or absence of soman (100 ug/kg, s.c.). Thirty min after injection, animals were killed by decapitation for ChE assay or by focused head microwave for ACh analysis, and brains were dissected into 6 parts: brainstem (B), cortex (C), hippocampus (H), midbrain (M), cerebellum (R), and striatum (S). Soman produced a 60% (S) to 80% (H, C) inhibition of ChE activity. The oximes, 2-PAM or HI-6, were unable to protect against soman-induced brain ChE inhibition, even though HI-6 treatment alone was beneficial in reversing the soman toxicity (Soc. Neurosci. Abstr. 10: 1184, 1984). Neither anticholinergics nor diazepam treatment affected ChE activity in any control brain area. Additionally, neither treatment reversed the ChE depression induced by soman. ACh levels were decreased by atropine sulfate and aprophen in all brain areas except B and R. In contrast, diazepam produced a dose-related increase of ACh in C, M and S. Therefore, the data suggest that atropine sulfate, aprophen, and diazepam changed brain ACh levels by a mechanism not related to ChE reactivation. Soman increased brain ACh levels by 6% (B) to 75% (C, H). Atropine sulfate did not reverse the soman-elevated ACh levels. Methyl atropine and oximes were not effective in modifying brain ACh levels of controls or in reversing the soman-induced ACh elevation. Although the relative ineffectiveness of these antidotes (except HI-6) in protecting rats against soman lethality may be related to their ineffectiveness in reversing the soman-induced brain ChE inhibition and ACh elevation, the observation of the effectiveness of HI-6 does not support such a relationship. Therefore, the measurement of brain regional ChE activity or ACh levels may not be useful in predicting the effectiveness of antidotes to soman poisoning. The study of other brain neurochemical functions of the cholinergic system, such as the turnover rate of ACh or the release of ACh from presynaptic terminals, may serve as a more specific indication of soman intoxication.

360.15 SIGNS OF SOMAN TOXICITY AND BRAIN REGIONAL CHOLINESTERASE ACTIVITY. V. Jimmerson, T.-M. Shih, T. Koviak*, O. Smith*, M. Pannella* and R. Mailman. USAMRICD, Aberdeen Proving Ground, MD 21010 & BSRC, Univ. N. Carolina, Chapel Hill, NC 27514

Soman, a potent organophosphate cholinesterase (ChE) inhibitor, reportedly depresses ChE activity in a dose-related manner in rat brain regions (Transact. Am. Soc. Neurochem., 14: 148, 1983). This relationship has been studied further to correlate inhibition of brain regional ChE activity (BRChE) with observable signs of organophosphate toxicity at a variety of soman doses. This correlation may prove useful in predicting BRChE in animal preparations where such activity cannot be measured directly, e.g., in brain tissue fixed by microwave irradiation. Male albino rats (200-275g) were injected with a single s.c. dose of 0.3, 0.5, 0.6, or 0.7 $LD_{50}$ of soman ($LD_{50}$=110 ug/kg) and were subsequently killed at selected time points. Animals were scored for toxic signs just prior to termination, according to the following "quality of life" (QL) categories: 0=sign free; 1=ataxia, muscle fasciculations and/or licking/chewing behavior; 2=salivation, limb weakness with hindlimb splaying, tremors, jerking and/or convulsions; and 3=moribund with loss of righting reflex. Occasionally, intermediate categories (i.e. $0^+$, $1^+$, $2^+$) were used. Toxic signs could usually be identified within 10 minutes after soman exposure. BRChE was assayed in brainstem (BST), cortex (CTX), hippocampus (HIP), midbrain (MB), cerebellum (CB) and striatum (STR) by the method of Groff et al (Clin. Toxicol., 9: 353, 1976) and is expressed as % of control activity (± standard error). At 0.3 $LD_{50}$ all animals (24/24) were free of signs of intoxication (QL=0). Similarly, at 0.5 $LD_{50}$, virtually all (28/30) animals were sign-free. At 0.3 $LD_{50}$ BRChE was minimally affected and showed 91±2%, 96±2%, 89±2%, 88±1%, 81±2%, and 103±5% of control ChE activity, whereas at 0.5 $LD_{50}$, BRChE was moderately affected and showed 70±3%, 65±3%, 59±4%, 69±3%, 62±4% and 91±2% in BST, CTX, HIP, MB, CB and STR, respectively. However, at 0.6 and 0.7 $LD_{50}$ recognizable signs of toxicity (QL=1 or above) were apparent in 52% (43/82) and 91% (29/32) of the animals, respectively. Furthermore, a correlation between QL and BRChE existed in each brain region examined. The coefficients of correlation (R) were 0.86, 0.77, 0.78, 0.87, 0.83 and 0.94 at 0.6 $LD_{50}$, and 0.70, 0.67, 0.63, 0.69, 0.65 and 0.71 at 0.7 $LD_{50}$ in BST, CTX, HIP, MB, CB and STR, respectively. Data for the 0.6 and 0.7 $LD_{50}$ doses were combined and the R for QL vs. BRChE were 0.82 (BST), 0.72 (CTX), 0.74 (HIP), 0.81 (MB), 0.78 (CB) and 0.84 (STR). Thus, we have demonstrated that in rats acutely exposed to soman at doses greater than 0.5 $LD_{50}$, various signs of intoxication may be observed and, regardless of dose, the magnitude of inhibition of BRChE correlates well with the severity of these recognizable toxic signs.

360.16 SKELETAL MUSCLE MYOPATHY FOLLOWING IN VITRO PYRIDOSTIGMINE EXPOSURE. C.S. Hudson*, D. Hinman* and M. Adler. Dept. of Pharmacol. & Exp. Thera., U of MD Sch. of Med., Balto., MD 21201 and Neurotoxicology Br., USAMRICD, APG, MD 21010.

Ultrastructural abnormalities have been reported in skeletal muscle after exposure to various carbamate and organophosphate cholinestrase inhibitors. The abnormalities appear to result from the consequences of acetylcholine accumulation, and evidence indicates that they are mediated, at least in part, by calcium influx through agonist sensitive endplate channels. The carbamate anticholinesterase agent pyridostigmine is used clinically for the treatment of myasthenia gravis and has been proposed as a pretreatment compound for protection against the toxic effects of organophosphorous cholinesterase inhibitors. Systemic administration of pyridostigmine has recently been shown to produce pathological alterations in rat skeletal muscle involving both pre- and postsynaptic components of the neuromuscular junction. Suggestions have been made that the pathology is dependent upon neurally evoked muscle activity. In order to determine the role of muscle activity on the ultrastructural lesions, we have examined the effects of pyridostigmine bromide under controlled in vitro conditions. Experiments were performed on rat phrenic nerve-diaphargm muscle preparations mounted in a twitch bath in Krebs-Ringer solution at 32°C and gassed with 95% $O_2$ - 5% $CO_2$; pH 7.4. The following stimulation paradigm was employed: (1) no stimulation; (2) indirect 20 Hz, 1 sec every 30 sec; (3) direct 20 Hz, 1 sec every 30 sec; (4) indirect 40/min. The stimulations were carried out for 2 hr in the absence or presence of 2 µM pyridostigmine. The muscles were fixed in 2.5% glutaraldehyde and processed for thin section electron microscopy. The presence of 2 µM pyridostigmine resulted in morphological alterations to the region of the neuromuscular junction regardless of the stimulation pattern employed. Presynaptic mitochondria were frequently swollen in experimental preparations. Pyridostigmine exposure also resulted in structural changes in subjunctional mitochondria ranging from rarefied areas of the matrix to extreme swelling of the organelles. Other subjunctional membrane bound organelles, including the sarcoplasmic reticulum and the nuclear envelope were usually swollen. Examination of the subjunctional sarcomeres revealed supercontraction sometimes to the point of complete disruption of myofibrillar organization. These alterations are similar to those which follow in vivo exposure to pyridostigmine. These results suggest that sustained neuronal activity is not required to produce the morphological alterations associated with pyridostigmine exposure. Supported in part by U.S. Army Contract DAMD-17-83-C-3126 to C.S.H.

360.17 ALUMINUM TOXICITY AND RAT CNS CHOLINERGIC ACTIVITY. Donald J. Connor and Richard S. Jope (Spon: John Monti), University of Alabama, Birmingham, AL 35294.

Recent reports indicate that aluminum is a neurotoxin in humans and animals. In human patients, aluminum has been implicated in the development of dialysis encephalopathy. Animal studies have shown that aluminum toxicity results in cognitive impairment and neuronal abnormalities, including neurofibrillary tangles, decreased synaptic density and dendritic debranching.

The effects of aluminum administration, especially dietary aluminum, on neurotransmission in the central nervous system are poorly understood. Since cholinergic activity is closely associated with learning and memory, we examined the effects of aluminum administration on several parameters of cholinergic function in rat brain. Three administration procedures were used:

1.) Acute dosage: aluminum lactate (600 or 1800 mg/kg body weight) via intragastric intubation.
2.) Subchronic dosage: aluminum lactate (1200 mg/kg body weight) via intragastric intubation on alternate days for a two week period.
3.) Chronic dosage: aluminum citrate ad lib in the drinking water at concentrations of 1% or 3% for 1 and 2 months. A chronic group given 2% aluminum citrate solution ad lib was injected with parathyroid hormone (i.p.) twice weekly for a one month period.

The acute and subchronic groups were sacrificed one, seven and twenty-eight days after intubation. The chronic groups were sacrificed one or twenty-eight days after the last aluminum administration. Several components of cholinergic function were measured in discrete brain regions. Choline acetyltransferase activity was determined in homogenates of striatum, hippocampus and cortex. Acetylcholine synthesis and release was measured in cortical slices using deuterium labelled choline as a tracer and quantitation by gas chromatography/mass spectrometry. The $B_{max}$ and apparent $K_D$ of muscarinic receptors were characterized by binding with tritiated 3-quinuclidinyl benzilate in homogenates of cortex, hippocampus and striatum. High affinity choline transport was measured in synaptosomes from cortex, hippocampus and striatum of chronically treated rats. Each of these processes was also measured in control tissue in the presence of aluminum added in vitro.

There were no significant effects of in vitro aluminum or acute in vivo administration of aluminum on any of these cholinergic processes. There were only minor effects of chronic treatment with aluminum on cholinergic processes.

360.18 αBUNGAROTOXIN NICOTINIC RECEPTOR AND NICOTINE-INDUCED SEIZURES. L.L. Miner*, M.J. Marks* and A.C. COLLINS*. (SPON: D.M. Gilliam). Institute for Behavioral Genetics, University of Colorado, Boulder, CO 80309.

The use of α-bungarotoxin (BTX) as a ligand to measure central nervous system nicotinic receptors has been questioned. The failure of BTX to inhibit neural function in the spinal cord and sympathetic ganglia of various species has led to questions concerning the physiological relevance of the BTX binding site in brain. Recently we have demonstrated a relationship between BTX binding in the hippocampus and sensitivity to nicotine-induced seizures in mice. In a previous study, we demonstrated that C3H/2Ibg mice are more sensitive to nicotine-induced seizures than are DBA/2Ibg mice. In addition, there are also differences in BTX binding in the hippocampus of these two strains, with C3H mice having greater binding. Utilizing a classical F2 and backcross genetic design, we demonstrated that animals which seize when given a 4.0 mg/kg dose of nicotine intraperitoneally (ip) have more BTX binding sites than those animals that do not seize. No differences were observed in $K_d$ or the displacement of BTX by L-nicotine. A genetic correlation, which indicates the extent to which two traits are influenced by the same gene or set of genes, between "seizure sensitivity" (whether an animal seizes or not) and hippocampal BTX binding was estimated (r = 0.21).

In order to obtain a quantitative measure of seizure sensitivity the following experiments were undertaken. Cannulae were implanted in the right jugular veins of 65 ± 10 day old mice from the C3H and DBA parental strains and their derived crosses (F1, F2 and backcrosses). After recovery from surgery, animals were infused with 2.0 mg/kg/min nicotine until a clonic seizure occurred. After the occurrence of a seizure, the animals were sacrificed, brains removed and dissected. Nicotinic receptors were measured with BTX as the ligand in three brain regions: cortex, midbrain and hippocampus. The pattern of inheritance for BTX binding was similar to that observed previously: C3H > F1 x C3H backcross = F2 > F1 = F1 x DBA backcross = DBA. In addition, C3H mice were more sensitive to the seizure inducing effects of nicotine than DBA mice. However, the pattern of inheritance was different from that found previously for mice given nicotine ip. The genetic correlation between hippocampal BTX binding and dose of nicotine received when a seizure occurred was greater (r = 0.32). To test further the relationship between seizure sensitivity and BTX binding in the hippocampus various other inbred strains (C57BL/6Ibg, BALB/cByL, A/Ibg, RIIIS/J, AKR/J and ISBI/Crg1) are being tested.

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360.19 CHARACTERIZATION OF A MODEL OF STATUS EPILEPTICUS IN RATS. R. Morrisett, R.S. Jope and O.C. Snead. University of Alabama at Birmingham, Birmingham, AL 35294.

We examined the seizure activity produced in rats by administration of the cholinergic agonist, pilocarpine, both alone and following a low dose of lithium (Honchar et al., Science 220, 327, 1983). Adult, male Sprague-Dawley rats were stereotaxically implanted with cortical and depth electrodes in the dorsal hippocampus, caudate nucleus and basolateral amygdala while under halothane anesthesia. After one week EEG recordings were made with the rats freely moving in heated plexiglass recording chambers.

Generalized convulsive status epilepticus occurred in 100% of rats treated with pilocarpine (30 mg/kg, sc) 24 hr following administration of lithium chloride (3 mEq/kg, i.p.). Signs of peripheral cholinergic stimulation were evident within 5 min of pilocarpine administration. During the next 15-20 min the rats displayed several behavioral automatisms, including wet-dog shakes, grooming, scratching and chewing. Prominent single spike activity appeared 20.3 ± 1.3 (SEM) min after pilocarpine treatment and was always associated with staring behavior. Spike activity rapidly progressed during the next 2-3 min, with rats displaying head bobbing movements, associated with spike trains, followed by rearing and forelimb clonus. The latency to tonic clonic seizure activity and forelimb clonus was 22.4 ± 1.2 min from the time of pilocarpine administration. During a period of 1-2 min the generalized spike activity was separated by intermitant low voltage activity after which the spike trains became continuous. Generalized convulsive status epilepticus occurred in every animal treated with this combination of lithium and pilocarpine. Once status epilepticus did occur, it continued unabated for several hours and resulted in death within 24 hr in 19 out of 20 rats.

By testing the effects of high doses of pilocarpine alone we determined that lithium potentiated the convulsant effect of pilocarpine by over 13-fold. Atropine administered 15 min prior to pilocarpine in lithium treated rats effectively blocked seizure activity.

Since diazepam is considered a drug of choice for treatment of status epilepticus in human patients, we tested its effects on status epilepticus produced by the lithium-pilocarpine treatment. Pretreatment with diazepam (5 mg/kg) totally blocked the development of paroxysmal spike activity and of status epilepticus in all rats tested and all rats survived the experiment. The animal model described here should be useful for the study of the pathogenesis and treatment of generalized convulsive status epilepticus.

360.20 INDUCTION OF A DESYNCHRONIZED (D) SLEEP-LIKE STATE BY CHOLINERGIC AGONISTS DEPENDS UPON MICROINJECTION SITE WITHIN THE PONTINE RETICULAR FORMATION (PRF). H.A.Baghdoyan*, R.W.McCarley and J.A.Hobson. Lab. of Neurophysiology, Harvard Medical School, 74 Fenwood Rd., Boston, MA 02115.

Direct administration of cholinergic agonists into the PRF produces a behavioral state (D-ACh) which is similar to D sleep. The present study demonstrated that: 1) the latency to onset and the total percentage of D-ACh are dependent upon the anatomical site of drug injection within the PRF, and 2) the percentage of D-ACh is correlated with the latency to onset of D-ACh .

Twelve adult, male cats were implanted with chronically indwelling guide tubes aimed at the pontine brainstem and standard polygraphic electrodes for measuring EEG, EOG, EMG, and PGO waves. A total of 41 microinjections of either carbachol (4ug) or a mixture of ACh (5 ug) and neostigmine (20 ug) were made into 20 different pontine sites, and 21 control (saline) injections were made into 11 different sites. Regression analyses were performed using the three dimensional coordinates of each injection site (posterior, vertical and lateral) as independent variables and the percentage and latency of D-ACh as dependent variables.

Following drug administration, the posterior coordinate of the injection site accounted for 31% of the variance in D-ACh latency ($p<0.001$) and 34% of the variance in D-ACh percentage ($p<0.001$). Injections into the more rostral sites (range: 1.8P to 7.2P) produced the shortest latencies and highest percentages. The vertical coordinate of the injection site accounted for 11% of the variance in D-ACh latency ($p<0.05$) and 38% of the variance in D-ACh percentage ($p<0.001$). Injections into the more dorsal sites (range: -5.5 to -10.0) produced the shortest D-ACh latencies and the highest D-ACh percentages. The lateral coordinate of the injection site (range: 0.3 to 1.9) did not have a significant influence on latency or percentage. Following saline injections there was no significant relationship between the anatomical coordinates of the injection site and D latency or D percentage.

The highest D-ACh percentages were significantly correlated with the shortest D-ACh latencies ($r=0.62$, $p<0.001$). Ninety-two percent of drug injection trials which induced D-ACh latencies under 20 min had D-ACh percentages greater than 50%. The relationship between latency and percentage was dose dependent; with low doses it was possible to reduce the latency to onset without increasing the percentage of D-ACh. Following saline injections there was no significant relationship between latency and percentage.

These findings support the hypothesis that D sleep is generated by an anatomically distributed, cholinoceptive neuronal system within the PRF.

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360.21 LESIONS OF THE NUCLEUS BASALIS MAGNOCELLULARIS (NBM). BIOCHEMICAL AND BEHAVIORAL STUDIES IN THE RAT. M. Le Moal, W. Mayo* and H. Simon* (SPON : C. Kordon). INSERM U-259 Psychobiologie des Comportements Adaptatifs, rue Camille Saint-Saëns, 33077 Bordeaux, France.

Since the demonstration of the degeneration of cholinergic cells of the NBM in patients suffering from senile dementia of Alzheimer type, these neurons have been subject of growing interest. We have studied in the rat the behavioral consequences of bilateral electrolytic (radio-frequency current) or ibotenic acid (7.5 µg) - (0.5 µl) lesions within the basal forebrain in the NBM nucleus region. Both types of lesions decreased choline acetyl transferase (CAT) activity in the anteromedial prefrontal cortex, in the sensorimotor cortex and in the amygdala. CAT activity was unchanged in the striatum and in the hippocampus. While electrolytic lesions destroyed dopaminergic and noradrenergic fibers innervating the prefrontal cortex region, the ibotenic acid injections spared these neurons and dopamine or noradrenaline were not significantly decreased in the cortex.

Electrolytic and ibotenic lesions led to profound disturbances in spontaneous and learned behaviors. Rats with lesions showed spontaneous locomotor hyperactivity, deficits in the exploratory responses, in feeding and in hoarding behaviors. These results are interpreted in terms of temporo-spatial integrative processing deficits.

Learned behaviors were also impaired as revealed by a poor performance observed in a T-maze, in a 8 arm radial maze and in a water maze. These results suggest perseverative responses and memory disorders.

Taken together, these data suggest that NBM lesions have important functional consequences similar to those generally attributed to hippocampal dysfunction. The specific involvement of cholinergic neurons in these cognitive dysfunctions remains to be elucidated.

## SPECIAL LECTURE

361 SPECIAL LECTURE. PATCH CLAMPING BEYOND NEURONS: ION CHANNELS IN LYMPHOCYTES AND OTHER NON-NEURONAL CELLS. M. Cahalan, University of California, Irvine.

The gigaohm seal patch clamp technique has allowed characterization of ion channels from diverse sources including plants, blood cells, epithelia, and glia. Distinct ion channel types from broadly divergent sources can be recognized on the basis of gating properties, ion selectivity, and pharmacological characterization. Channels that have long been associated with membrane excitability of nerve and muscle have recently been found in blood cells. Within the immune system sodium, calcium, and several types of potassium channels have been discovered. T lymphocytes express predominantly voltage-dependent potassium channels with properties very similar to delayed rectifier potassium channels in nerve cell bodies and skeletal muscle. A basophilic leukemia cell line and macrophages express inward rectifier potassium channels. Delayed rectifier and calcium-activated potassium channels can also be expressed in macrophages. Voltage-dependent sodium channels blockable by tetrodotoxin have been seen in T-lymphocytes, an erythroleukemic cell line and natural killer cells. Calcium channels have been discovered in B-cell hybridomas. In several instances the density of ion channels expressed can be modulated by varying the culture conditions or by stimulating the cells to divide. For example, the surface density of potassium channels in mouse T lymphocytes increases by more than an order of magnitude 24 hours after mitogen stimulation. Potassium channel expression within the T cell lineage also appears to change developmentally. Resting splenic or lymph node mouse T-cells express few potassium channels, while many more are expressed in thymocytes, from which mature T-cells are derived. The potassium channels are required for mitogenic activation. Blocking the channels with a variety of channel blockers inhibits protein synthesis, DNA synthesis, and secretion of T-cell growth factor, interleukin-2. The gating properties of the potassium channel are modulated immediately upon addition of mitogenic lectins, increasing the probability that potassium channels will be open. The possible functional role of channels in secretion, intracellular signalling, and cell division will be discussed.

362 WORKSHOP. ESTROGEN AND THE NIGROSTRIATAL DOPAMINE SYSTEM. J.B. Becker, The University of Michigan (Chairperson); P.J. Bedard, Hospital Enfant Jesus, Quebec City; L.A. Chiodo, Sinai Hospital, Detroit; J.H. Gordon, The Chicago Medical School; R.E. Hruska, SUNY-Buffalo; C.J. VanHartesveldt, University of Florida.

The idea that estrogen can influence neural activity in the hypothalamus and limbic system is now well accepted. In addition, considerable evidence has accumulated to indicate that estrogen modulates neural activity in other areas of the brain. Clinical reports of chorea graviderum and chorea associated with oral contraceptives suggested that neural activity in the basal ganglia could be influenced by ovarian hormones. Subsequent research in nonhuman animals has demonstrated that behavioral indices of nigrostriatal dopamine (DA) activity are profoundly influenced by the ovarian hormones, and by estrogen in particular. In this workshop we will discuss: (1) the variables that influence the effect(s) of estrogen on striatal DA activity, and (2) the mechanism(s) of estrogen's action on the striatal DA system.

Dr. Gordon will show that estrogen has a multiphasic effect on striatal DA receptor sensitivity. There is an initial hyposensitivity that is mimicked by incubating striatal tissue with estradiol, and a delayed hypersensitivity that may be induced by the formation of catechol estrogens from estradiol. Dr. Hruska will discuss the role of prolactin in the estrogen-induced hypersensitivity of postsynaptic striatal DA receptors. In addition, he will present evidence that estrogen produces behavioral effects that are not mediated by estrogen-induced changes in postsynaptic striatal DA receptors or by estrogen-induced release of prolactin.

Dr. Chiodo will describe the effect of estrogen on electrophysiological indices of nigrostriatal DA activity. Estrogen induces a rapid decrease in the spontaneous activity of substantia nigra zona compacta DA cells and this effect is blocked by DA antagonists or the inhibition of protein synthesis. Dr. Becker will present evidence that estrogen potentiates striatal DA release and amphetamine-induced rotational behavior. Dr. VanHartesveldt will present the results from behavioral studies that demonstrate that the direct application of estrogen to the striatum has rapid effects on striatal DA-mediated behaviors. Finally, Dr. Bedard will present the results of experiments investigating the effectiveness of estrogen therapy in the treatment of tardive dyskinesia. He will also discuss evidence that the dose of estrogen administered influences both the behavioral and the neurochemical consequences of estrogen treatment.

The workshop will conclude with a roundtable discussion of the information presented and recommendations for future research in the area.

363 SYMPOSIUM. SENSORY MECHANISMS IN SYMPATHETIC GANGLIA. D.L. Kreulen, Univ. of Arizona (Chairperson); J.H. Szurszewski, Mayo Foundation; M. Schultzberg*. Karolinska Institutet; Z.J. Bosnjak, Med. Col. of Wisconsin; J. Krier, Michigan State Univ.

Studies in recent years have demonstrated gastrointestinal, cardiovascular and pulmonary sensory inputs to ganglion cells in several sympathetic ganglia. Immunohistochemical studies on the localization of neuropeptides have demonstrated that both primary sensory neurons and neurons within the viscera project to some sympathetic ganglia. In this symposium we will present the evidence for the existence of sensory pathways, discuss the role of cholinergic and noncholinergic synaptic transmission in these pathways and discuss the function of peripheral ganglionic reflex arcs in the autonomic control of the viscera.

Joseph Szurszewski will describe the original discovery of peripheral mechanosensory input to abdominal sympathetic ganglia. His studies on the convergence of peripheral synaptic inputs to prevertebral ganglionic neurons revitalized the concept of sympathetic ganglia as peripheral reflex centers.

Marianne Schultzberg will describe the immunohistochemical localization of various neuropeptides in sympathetic ganglia emphasizing their localization in sensory pathways. Peptide-containing nerve fibers project to sympathetic ganglia in primary sensory pathways and in "peripheral sensory pathways" that project from the gastrointestinal tract to abdominal sympathetic ganglia.

Zeljko Bosnjak will discuss sensory mechanisms in thoracic sympathetic ganglia. Recent evidence indicates that these ganglia contain the structural and functional elements necessary for neural integration. In the stellate ganglion, efferent nerve activity can be modified by pulmonary and cardiovascular afferent input providing the substrate for peripheral reflexes in these ganglia.

Jack Krier will discuss the visceral connections of neurons in lumbar paravertebral sympathetic ganglia. Not only do these ganglia provide innervation to the abdominal viscera, but a population of the neurons in the ganglia receive synaptic input from fibers that come from the innervated organs. Synaptic inputs to the ganglia utilize both cholinergic and noncholinergic mechanisms.

David Kreulen will describe the noncholinergic or putative peptidergic sensory pathways from the gastrointestinal tract and mesenteric blood vessels to the abdominal prevertebral ganglia. Activation of mechanosensory inputs by distension of either the intestine or the blood vessels results in slow synaptic potentials. The simultaneous activation of cholinergic and noncholinergic sensory pathways suggests that acetylcholine and peptide neurotransmitters are simultaneously released on to these sympathetic neurons.

## SENSORY SYSTEMS: VISUAL CORTEX V

364.1 FLATTENING CORTEX: AN OPTIMAL COMPUTER ALGORITHM AND COMPARISONS WITH PHYSICAL FLATTENING OF THE OPERCULAR SURFACE OF STRIATE CORTEX. E. Schwartz and B. Merker. Brain Res. NYU Med. Ctr. 550 1st Ave NY NY 10016 and Courant Inst. of Math. Sciences.

Recent progress in functional mapping methods, particularly with respect to striate cortex topography, have extended the precision of measurement to the point where errors introduced by the curvature of the cortical surface should be taken into account. Furthermore, the study of any brain surface in which tangential architectural patterns are of interest would greatly benefit by the availability of workable methods of computer aided anatomical display.

We have developed an algorithm which optimally flattens brain surfaces. The geometric aspect of the 3D brain surface which is optimally preserved is its matrix of inter-point distances. We use a variational (hill-climbing) algorithm, together with an algorithm which obtains minimal geodesic distances (in the brain surface). The result is a (near) isometric mapping of the three dimensional cortical surface into the plane, together with a map of the error introduced in this process.

We illustrate this method with examples of macaque striate cortex, in the form of a three-dimensional computer graphics model reconstructed from serial sections. The 3D cortical model, and its flattened representation are used to generate a map of distance error produced by flattening. This error map will be discussed in the context of the measurements of mean and gaussian curvature of the same tissue surface (Merker and Schwartz, ARVO , 26 :164,1985).

In addition, we obtained measurements of error introduced by physically flattening similar samples of macaque striate cortex. The physical flattening was performed by squeezing unfixed, dissected opercular cortex (with pia removed) between glass slides moved in a micromanipulator assembly. The cortex was marked by a grid of india-ink dots, and movie films were taken as the cortex was pressed and relaxed. Sequential movie frames preserve the relative displacement of grid dots, allowing a map of error introduced in flat mounting to be constructed. These maps will be compared with the results of computer flattening described above.

This joint application of neuroanatomy, image processing, computer solids modeling, and differential geometry has wide applicability. Besides providing an understanding of the errors introduced in flattening cortex, it forms an essential part of the general computer aided characterization of cortical surfaces and their embedded functional architectures.

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364.2 COMPUTATIONAL ALGORITHMS FOR THE PRODUCTION OF TWO DIMENSIONAL MAPS OF CEREBRAL CORTEX. G. J. Carman and D. C. Van Essen. California Institute of Technology, Pasadena, CA 91125.

In order to improve upon existing manual techniques for the construction of two dimensional, unfolded representations of the cerebral cortex (Van Essen and Maunsell, J. Comp. Neurol. 191: 255-281, 1980), we are developing a set of computational algorithms which will allow for the production of such maps by computer. Our procedures use regularly spaced histological sections to create a lattice of points which serves as a three-dimensional reference surface. For each point on this reference surface, a corresponding point is created on a two-dimensional map surface in a fashion which preserves topology. A metric of local distortion termed the energy is determined for each point from a comparison of the local geometry on the map with the corresponding geometry on the reference surface. The sum of energies of all points yields a metric of global distortion that is minimized in order to generate an optimal map of the reference surface.

To find this global energy minimum, we subject the map to simulated annealing (Kirkpatrick et al., Science 220: 671-680, 1983). In our implementation of this iterative algorithm, random displacements of points are attempted within the plane of the map. Displacements resulting in a more accurate local geometry (decrease in energy) are always accepted, while those resulting in a less accurate local geometry (increase in energy) are accepted with a probability dependent upon a control parameter termed the temperature. Initially, the temperature is set high to allow positions of points on the map to become randomized. The temperature is then gradually reduced over successive iterations, allowing the points to approach their optimal local geometries and resulting in the emergence of an optimal global conformation of the map.

These procedures have demonstrated their ability to maintain topology while generating minimally distorted two-dimensional maps of three-dimensional mathematical surfaces. We are extending these procedures to the mapping of large regions of visual cortex of the macaque monkey. Although certain aspects of the implementation remain to be developed, the results obtained thus far demonstrate that these algorithms provide a practical means for the computation of unfolded, two-dimensional maps of the cortex. (Supported by NRSA 2 T32 GM07737.)

364.3 PATCHY INTEGRATION OF GENICULATE INPUTS IN CAT LATERAL SUPRASYLVIAN VISUAL CORTEX. C. Lee, J.G. Malpeli and T.G. Weyand. Dept. of Psychology, Univ. of Illinois, Champaign, IL 61820.

We have examined the contributions of two subdivisions of the lateral geniculate body to neuronal activity in cat posteromedial lateral suprasylvian cortex (PLMS). Layer A of the dorsal lateral geniculate nucleus and the medial interlaminar nucleus (MIN) were reversibly inactivated with 115 nl injections of 4 mM cobaltous chloride and 2% lidocaine hydrochloride, respectively. For 105 histologically verified sites in the PMLS of 5 anesthetized, paralyzed cats, we determined the effect on the visually driven activity of single cells and multiple-unit clusters for inactivation of either layer A or the MIN. At 25 sites, both manipulations were done on the same cortical cells.

For inactivation of either layer A or the MIN, the effect on cortical activity was variable, ranging from total elimination of activity to slight enhancement of the visually evoked response. There was a strong tendency for the effects to occur in clusters with a reciprocal relationship between the contributions of layer A and the MIN to cortical activity. For neither layer A nor the MIN were these effects strictly related to cortical layer, suggesting that there are interdigitating columns or patches cutting across layers that are under selective control of individual geniculate subdivisions. We have begun to investigate the effects of inactivating layer C of the lateral geniculate nucleus on the PMLS, and preliminary results indicate that there are also clusters that are strongly dominated by layer C input.

The contribution of layer A to the PMLS is probably mediated by either areas 17 or 18, the main targets of layer A. The projections from areas 17 and 18 to the PMLS arise from supragranular layers (Gilbert and Kelly, J. Comp. Neurol., 163: 81, 1975). Inactivating layer A has little effect on supragranular layers in area 17 (Malpeli, J. Neurophysiol., 49: 595, 1983). On the other hand, we have observed that inactivating layer A does silence many cells in supragranular layers of area 18. Therefore, it is likely that the effects in the PMLS of inactivating layer A are mediated via area 18. We propose that the clusters of layer A dependent cells in PMLS are recipients of the patchy projections from area 18 to PMLS that have been observed anatomically (Symonds and Rosenquist, J. Comp. Neurol., 229: 1, 1984).

Previous work from this laboratory has shown that the contributions of geniculate layer A to cortical activity in area 17 are in general not horizontally clustered but are laminar specific (Malpeli, J. Neurophysiol., 49: 595, 1983). The present results indicate that different areas of visual cortex are organized in a fundamentally different way with respect to their pattern of integrating thalamic inputs. (This research was supported by NIH grants RO1 EY32114, T32 EY07005, K04 EY00229, and grants from the University of Illinois Research Board).

364.4 RETINOTOPIC ORGANIZATION IN OUTLYING VISUAL AREAS OF CAT CEREBRAL CORTEX AS REVEALED BY ANALYSIS OF CORTICOTECTAL TOPOGRAPHY. C.R. Olson and T.M. Farrell*
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The aim of these experiments was to reveal and characterize retinotopic organization in cortical areas projecting to cat superior colliculus. Our approach was to analyze the distribution of cortical cells labeled retrogradely with tracers deposited in the superior colliculus at particular retinotopic loci. We report here the results of four experiments in which one retrograde tracer (nuclear yellow; NY) was placed in medial superior colliculus (at a site representing the superior visual field) and a second tracer (bisbenzimide; Bb) was placed in lateral superior colliculus (at a site representing the inferior visual field).

Within areas 17, 18, 19, 21a, 21b, 20a and 20b, labeling patterns conformed to standard electrophysiological maps: NY and Bb labeled complementary populations of cells, with Bb concentrated in two zones known to represent the lower visual field, one spanning anterior parts of areas 17, 18, 19 and 21a and the second straddling areas 20a and 20b.

Within both banks of the middle suprasylvian sulcus, transported label was present. Cells labeled with NY and Bb formed largely nonoverlapping populations. Cells labeled with Bb (from lower-visual-field colliculus) had a generally more medial location than cells labeled with NY.

In several cortical areas not previously characterized with regard to retinotopy, cells labeled with NY and Bb were largely or partially segregated. In area 7, Bb (the lower-visual-field tracer) was concentrated in the lateral bank of the lateral sulcus and on immediately adjacent gyral cortex, whereas NY-labeling was heaviest on the crown of the suprasylvian gyrus. In the medial frontal eye field, cells labeled with Bb from the lower-field injection were concentrated in two patches on the ventral bank of the cruciate sulcus; NY-labeled cells were distributed in complementary fashion within the sulcus and appeared to extend farther onto the medial face of the frontal pole. In the ectosylvian visual area (EVA), cells labeled with Bb and NY formed a complex system of alternating patches. Segregation of cells labeled with Bb and NY was also observed in a cortical zone encompassing the entire anterior bank of the posterior suprasylvian sulcus and the immediately adjacent cortex on the posterior ectosylvian gyrus.

In summary, by analyzing corticotectal topography with multiple tracers we have revealed previously unsuspected patterns of retinotopic organization in several outlying visual areas of the cat's cerebral cortex.

364.5 VISUAL TOPOGRAPHY OF CORTICAL PROJECTIONS TO MONKEY SUPERIOR COLLICULUS. C.L. Colby and C.R. Olson
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The superior colliculus (SC) receives input from prestriate visual cortex, the intraparietal and superior temporal sulci and prefrontal cortex. We took advantage of the precise visual topography in the superior colliculus to delineate the organization of the visual field representation in these zones.

Distinguishable retrograde fluorescent tracers were injected into the upper and lower visual field representations in the SC of 3 macaques. The extent and topographic location of each injection were determined by examining the distribution of labeled cells in V1.

In prestriate cortical areas V2, V3, V3A, V4 and PO, substantial retrograde label was observed in layer V. Cells labeled from the upper and lower field colliculus injections were segregated. Within each visual area, the arrangement of labeled cells was consistent with the known retinotopic organization of that area.

In the intraparietal sulcus (IPS), moderate labeling was observed in the lateral bank and sparse labeling in the medial bank. In each bank there was clear segregation of cells labeled from the upper and lower field injections. Very few labeled cells were observed on the convexity of the inferior parietal lobule.

Substantial labeling was observed in the caudal superior temporal sulcus (STS). Area MT, identified in adjacent myelin-stained sections, was heavily labeled in a pattern concordant with its known visual topography. Outside of MT, there was clear segregation of cells labeled from the upper and lower field injections in a number of distinct regions. In the rostral half of STS, labeling was relatively sparse and cells labeled from either of the two injections were largely intermingled.

In prefrontal cortex, there was substantial labeling in a zone encompassing the frontal eye fields and the principal sulcus and extending onto the medial surface. Within the anterior bank of arcuate sulcus, cells labeled from the lower field injection were found in discrete patches confined to the inferior limb, while cells labeled from the upper field injection were distributed throughout both limbs.

These results suggest that there are additional retinotopically organized cortical regions outside of the currently known visual areas.

364.6 DIMENSIONAL ATTENTION EFFECTS IN THE RESPONSES OF V4 NEURONS OF THE MACAQUE MONKEY. Shaul Hochstein and John H.R. Maunsell.
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When performing a matching task in which a single dimension is varied and other dimensions are fixed, attention must be paid to the variable dimension while other stimulus dimensions may, or even should, be ignored. A considerable improvement in performance is expected if irrelevant dimensions are not discriminated. On the single neuron level, this dimensional attention may correspond to a sharpening of the tuning curve to the attended dimension and a concurrent flattening out of the tuning curves of the irrelevant dimensions. We looked for such attentional effects in V4 neurons of the alert monkey trained to respond to a match in one of two stimulus dimensions: either the color or the orientation of a visual grating stimulus.

Electrophysiological recordings were made from single units in rhesus cortical area V4 during the performance of a sequential visual matching task. The monkey had to respond upon seeing a stimulus which contained the value of a dimension which was also present in a preceding cue presentation. The cue also served to inform the monkey which dimension was to be varied in the subsequent series of stimuli from which the match had to be selected. For example, if the cue was a small red patch, the series of stimulus gratings were of a single orientation but varied in color and the monkey had to respond to the red grating. If the cue was a black and white vertical grating, the series of grating stimuli were of a single color but varied in orientation and the monkey had to respond to the vertical colored grating. In this way it was possible to determine how the response of a neuron to various combinations of orientation and color depended upon the dimension to which the monkey was attending. About 1/3 of the units studied in detail showed strong dependences on dimensional attention. The changes found in the responses were consistent with the hypothesis that tuning curves are generally sharpened to the dimension being attended.

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364.7 SELECTIVE ATTENTION GATES VISUAL PROCESSING IN AREA V4 AND THE INFERIOR TEMPORAL CORTEX OF THE MACAQUE. J. Moran* and R. Desimone (SPON: L. Raskin). Laboratory of Neuropsychology, NIMH, Bethesda, MD 20205.

As one moves from V1, though prestriate cortex into inferior temporal (IT) cortex, receptive fields (RFs) increase dramatically in size. Normally, many different stimuli will fall within these large extrastriate RFs, and an IT RF may even encompass an entire scene. How then does the visual system extract information about the properties of individual stimuli at specific locations? Our results indicate that when a monkey attends to a location within the RF of an extrastriate neuron, responses to stimuli at other locations within the RF are greatly attenuated, and, thus, the neuron only communicates the properties of the stimulus at the attended location. This filtering of unwanted information may also explain why we are aware of so few of the stimuli on our retinas at any one time.

The general strategy of the experiment was as follows. While the monkey held a bar and gazed at a fixation spot, a stimulus appeared briefly at one location followed shortly by a second, briefly presented stimulus at the same location. The monkey was rewarded for releasing the bar immediately if the two stimuli matched and for releasing the bar after a fixed delay if they did not match. Two other, irrelevant stimuli were also presented on each trial, each concurrent with one of the stimuli used in the task. For 8 trials the animal attended to the stimuli at one location and then, after a cue, switched its attention to the stimuli at the other location for 8 trials. The location of the animal's attention was repeatedly switched back and forth. Thus, identical sensory conditions were maintained across blocks of 8 trials but the locus of the animal's attention varied.

In two rhesus monkeys 363 cells were recorded from IT cortex, 181 from V4, and 28 from V1. When both attended and ignored stimuli were within the RF of a cell in area V4 or IT cortex, the response of the cell was determined by the attended stimulus; the influence of the unattended stimulus upon the cell was greatly attenuated. For example, if a cell was selective for red stimuli, it would respond well if a red stimulus appeared at an attended location but poorly or not at all if a red stimulus appeared at an ignored location. The RFs of cells in V1 were so small that, when relevant and irrelevant stimuli were placed within them, the monkey could not perform the task, i.e. could not ignore the irrelevant stimuli.

Cells in both V4 and V1 responded the same to a RF stimulus regardless of whether the monkey attended to it or to another stimulus outside the RF. The RFs of cells in IT cortex were too large to position a stimulus outside of them.

364.8 MECHANISMS FOR SELECTIVE ATTENTION IN AREA V4 AND INFERIOR TEMPORAL CORTEX OF THE MACAQUE. R. Desimone and J. Moran.* Laboratory of Neuropsychology, NIMH, Bethesda, MD 20205.

In a companion study we found that when a monkey selectively attends to a location within the receptive field (RF) of a V4 or IT neuron, the responses of the neuron to stimuli at unattended locations within the RF are greatly attenuated or suppressed. In the present study, we investigated the mechanism by which attention gates V4 and IT neural responses and how this mechanism interacts with other non-sensory influences on the cells.

The monkeys performed the same basic task as in the companion study. The results indicate, first, that the suppressive mechanism has a very high spatial resolution. The responses of cells to an ignored stimulus could be suppressed if the latter was separated by as little as 1/3° from the attended stimulus within the RF. Second, the suppressive mechanism does not act chronically to suppress responses to stimuli at ignored locations, but rather is driven or triggered by the onset of the attended stimulus. The visual latency of the suppressive mechanism appeared to be 100 msec in V4, since shorter latency (75-100 msec) responses in V4 to ignored stimuli were unaffected until 100 msec after stimulus onset, at which point they were sharply cut off. Furthermore, if on a given trial the normally attended stimulus was not presented, the response to the normally ignored stimulus was not suppressed. Third, novel events can also negate or switch the suppressive mechanism, as the responses to unexpected stimuli within the RF were not suppressed. Fourth, attention cannot override the silent suppressive surrounds of V4 cells. The responses of V4 cells to a RF stimulus were reduced by a large bar of the same color in the surround, even when the animal attended to the RF stimulus. Fifth, in addition to spatially directed attention, other behavior-related mechanisms influence extrastriate cells. For example, independent of the locus of the animal's attention, many cells gave a small discharge preceding the animal's motor response, even with no stimulus present, and many had reduced activity when the animal was required to delay or withhold its response. It is not yet clear if these cells are the source or recipient of this motor signal, but in either case the signal may play a role in triggering the animal's response.

We suggest that the neurons that suppress extrastriate responses to ignored stimuli are located outside of V4 and IT, that they receive visual inputs either from V4 or in parallel with V4, that it takes at least 100 msec from the onset of a stimulus to suppress extrastriate responses, and that the attention mechanism itself can be switched by novel events. Finally, this mechanism for spatial attention appears to coexist with other behavior-related mechanisms within extrastriate cortex.

364.9 VISUAL SELECTIVE ATTENTION MODIFIES SINGLE UNIT ACTIVITY IN INFERIOR TEMPORAL CORTEX. Hedva Spitzer* and Barry J. Richmond. Laboratory of Neuropsychology, NIMH, Bethesda, 20205.

In the monkey, evoked potentials from inferior temporal (IT) cortex to a particular compound visual stimulus vary in their form depending on which dimension of the stimulus, its color or its shape, is being used by the animal to discriminate that stimulus from another(1). We tested whether such selective attention would alter activity at the neuronal level(2). We recorded from single units in area TE of IT cortex from two adult monkeys while they performed discrimination tasks along two dimensions: shape and texture. In each trial, while the monkey maintained fixation on a spot of light, one of four peripherally located compound visual stimuli was randomly presented on a screen. The stimuli were either squares or circles and had textures of either dots or stripes. If shape was the relevant dimension, the monkey was rewarded for an immediate behavioral response to the onset of one shape, e.g. a circle, and with a delayed behavioral response to the onset of the other, in this case a square, regardless of the texture. Similarly, if the texture was the relevant dimension, the monkey was rewarded for an immediate behavioral response to the onset of one texture, e.g. dots, and with a delayed response to the onset of the other, in this case, stripes, regardless of the shape.

Forty-eight percent (25/52) of the IT neurons recorded thus far responded differently ($p < .05$) to the same compound visual stimulus depending on which of its dimensions the animal was attending to. For each cell, we determined which of the four compound stimuli yielded the largest difference in response between the texture and shape discrimination trials, and which of these two types of trials yielded the larger response. We then identified that feature in the relevant dimension for the compound stimulus and checked whether the second compound stimulus with this same feature also elicited a larger response when the dimension to which it belonged was relevant. Ninety-one percent of IT cells showed this phenomenon. The results demonstrate that neuronal responses of IT cortex to a stimulus are modulated by attention to different aspects of that stimulus.

1. Nuwer and Pribram. Electroenceph. clin. Neurophys. 46 : 389-400 (1979).

2. Braitman. Br. Res. 307 :17-28 (1984).

364.10 TRANSMISSION OF INFORMATION BY NEURONS IN PRIMATE INFERIOR TEMPORAL CORTEX. Barry J. Richmond and Lance M. Optican* (SPON. F. Miles). National Institute of Mental Health and National Eye Institute, NIH, Bethesda, MD 20205.

Lesions of inferior temporal (IT) cortex degrade primate performance in pattern recognition tasks. Single-units in IT cortex show differential responses to various complex patterns (e.g., bars, brushes, faces), which have been characterized by univariate response measures (e.g., spike count). Often, the temporal distribution, as well as the number of spikes, varies in response to different stimuli. We asked if this temporal modulation was carrying information about stimulus parameters.

Shannon's Information Theory can quantify the amount of information transmitted through a neuron by measuring how much a response reduces the uncertainty about which stimulus was presented. We recorded single-units in IT cortex of four rhesus monkeys. To obtain a wide range of responses we presented a set of 128 stimuli based on orthogonal, two-dimensional Walsh functions. While the monkey fixated, the stimuli (each 40 degrees square and centered on the fixation point) were flashed on individually for 400 ms. The spike density, a measure of the response, was defined as the convolution of the spike train with a Gaussian pulse. Spike densities were quantified by principal component (PC) decomposition. The PC's were derived independently for each cell, yet all of the sets of PCs from 21 cells fell (about equally) into just two groups, according to whether the first PC was sustained or transient. (A cell in either group could show a sustained or transient response to an individual stimulus, so the grouping of PCs reflects the characteristics of the neuron's average response to a large number of stimuli.) The coefficients of the first three to four PCs were dependent upon the stimulus (bootstrap test, $p < 0.05$). Hence, the temporal modulation of the spike train, as well as spike count, is determined by stimulus parameters.

To quantify the information transmitted by neurons we measured the responses using either spike count alone (to form a count code), or the first three PCs together (to form a three-dimensional temporal code). Average information transmitted by the count code was $0.43 \pm 0.04$ bits (SE, $N = 21$), and by the temporal code was $0.88 \pm 0.05$ bits. Although the spike count can transmit as much as half the information in the temporal code, the spike count itself is not correlated with information ($r = 0.18 \pm 0.04$). Hence, a multidimensional spatial to temporal transform is needed to completely represent the ability of IT neurons to transmit information about stimulus parameters. Since all the sets of PCs fell into just two groups, we infer that a universal temporal code for representing stimulus features may exist.

364.11 **TWO TOPOGRAPHICALLY ORGANIZED VISUAL AREAS IN VENTRAL EXTRASTRIATE CORTEX OF THE MACAQUE MONKEY.** D. J. Felleman, E. A. DeYoe, and D. C. Van Essen. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

Ventral extrastriate cortex in the macaque contains a large region of cortex sandwiched between area VP posteriorly and the posterior inferotemporal area (PIT) anteriorly. Previous work from this laboratory has shown that at least part of this region receives inputs from ventral V2, but whether it should be considered as part of V4 or as one more separate areas has remained uncertain. To address this issue, we have employed electrophysiological mapping techniques and studies of cortical connections to assess the organization of this region of cortex.

We mapped the representation of visual space in cortex anterior to VP in both the inferior occipital (IOS) and occipito-temporal (OTS) sulci in 31 microelectrode penetrations in 4 hemispheres, covering a range of eccentricities from 1-20 degrees. The results indicate that there are two topographically organized representations of the superior contralateral quadrant in the cortex anterior to VP. The first region adjoins VP along the representation of the superior vertical meridian. Provisionally, we have named this region VA, the ventral anterior area, and suggest that it is probably distinct from V4. In front of it is a second region which shares a representation of the horizontal meridian with VA and contains a representation of the superior vertical meridian at or near the posterior border of PIT. Provisionally, we have termed this region, PT, the posterior temporal visual area. PT may correspond to the region of cortex identified as TEO by von Bonin and Bailey on the basis of cortical architecture. Each region has central fields located laterally, in the IOS, and peripheral fields located medially, in the OTS.

We used a double retrograde labelling technique to study the cortical connections of VA and V4 in the same hemisphere. Both injections produced clear stripe-like labelling patterns in V2 which were generally oriented orthogonal to the V1 border. In addition to connections to several well known visual areas, these injections produced interdigitating patterns of label in the intraparietal sulcus and across large portions of posterior inferotemporal cortex. Both the VA and V4 injections produced widely separated foci of label in PIT, suggesting that it contains multiple subdivisions. Furthermore, these patches of label were almost exclusively non-overlapping, which suggests a crude topographic organization within PIT. This result contrasts with previous single unit recordings from PIT in which most receptive fields included both upper and lower fields.

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364.12 DIVERGENT SIGNALS ENCODED BY NEURONS IN EXTRASTRIATE AREAS MT AND MST DURING SMOOTH PURSUIT EYE MOVEMENTS. R.H. Wurtz and W.T. Newsome. Laboratory of Sensorimotor Research, National Eye Institute, Bethesda, MD, 20205, and Dept. of Neurobiology & Behavior, SUNY at Stony Brook, Stony Brook, NY 11794.

We have studied the responses of single neurons in extrastriate areas MT and MST of monkey visual cortex during smooth pursuit eye movements. Both areas contain visually responsive neurons that are selective for the direction of motion of stimuli within their receptive fields. We have previously reported that some neurons in MST also discharge during foveal pursuit of a small, moving target in an otherwise dark room. The responses of such "tracking neurons" during pursuit cannot be explained by their passive visual properties and are therefore likely to be related to some aspect of the eye movement itself. In contrast, we observed no pursuit-related responses for MT neurons with eccentric receptive fields. In recent experiments, however, we have found that neurons within the foveal representation of MT often respond during pursuit of visual targets moving in a restricted range of directions. Such responses could be due to the "slip" motion of the target on the foveal receptive field that occurs when the speed of the pursuit eye movement is imperfectly matched to the speed of the target, or the responses could be related to some aspect of the eye movement itself. To test these alternatives, we studied the responses of foveal MT neurons and of tracking cells in MST while the monkey pursued an image that had been stabilized on the retina so as to eliminate target "slip".

Rhesus monkeys were trained to pursue small foveal targets moving at $16^{o}$/sec across a tangent screen. The monkeys were rewarded for keeping their eyes within an electronically defined window centered on the target. A trial began with a 600 msec interval of normal pursuit, and the target was then stabilized on the retina by using the monkey's eye position (measured with a magnetic search coil) to drive the pursuit target. The monkey pursued the target in this "open-loop" condition for 800 msec, and then normal pursuit conditions were restored for 400 msec before the trial was terminated. We found that the large majority of neurons in foveal MT ceased responding during the open-loop interval although they gave vigorous bursts during the intervals of normal pursuit. In contrast, tracking cells in MST responded during both conditions. These results suggest that neurons in foveal MT may encode the slip of the visual target during pursuit - information that may be useful in adjusting the gain of the pursuit movement. Tracking neurons in MST, on the other hand, encode a different aspect of the pursuit eye movement, possibly providing an internal representation of eye velocity during pursuit.

364.13 REMOVAL OF STRIATE CORTEX DOES NOT ABOLISH RESPONSIVENESS OF NEURONS IN VISUAL AREA MT OF THE MACAQUE. H.R. Rodman*, C.G. Gross, and T.D. Albright. Dept. of Psychology, Princeton Univ., Princeton, NJ 08544.

Area MT receives strong projections from striate cortex and from V2, which is dependent upon striate for visual responsiveness. Thus, the visual properties of MT neurons have been thought to reflect the further processing of its striate input. However, MT also receives inputs from other visual cortical areas and from the superior colliculus via the pulvinar. We report here that striate removal or inactivation does not abolish the responsiveness, direction selectivity or binocularity of the majority of MT neurons.

The central representation in striate cortex was removed unilaterally in one animal and bilaterally in a second. Six weeks later, repeated recording sessions were begun. Animals were immobilized and anesthetized with $N_2O$. Over 200 single- and multi-units in MT were tested for responsiveness and direction selectivity using spots and slits of light and dark edges. Within the portion of MT visuotopically correspondent with the striate lesion, responses were frequently weak and single neurons difficult to isolate. Nonetheless, over two-thirds of the units gave clear responses to an optimal visual stimulus. These units showed direction preferences and binocular responses similar to those found in the normal animal. Responses in portions of MT not representing the affected part of the visual field were essentially normal.

In the unilateral case, within the region corresponding to the striate lesion, most of the MT neurons with completely normal properties had receptive fields bordering or overlapping the vertical meridian. This phenomenon was not seen in the bilaterally lesioned animal, suggesting, in the unilateral case, a functional input from the contralateral (intact) striate cortex, possibly via the contralateral MT.

In a third animal, single unit recordings were made in MT during reversible cooling of dorsolateral striate cortex. The results were in agreement with those of the lesion experiments.

In summary, MT seems to contain a heterogeneous population of cells with respect to functional dependence on striate cortex, with many MT neurons retaining their properties in the absence of striate input. Recordings made in one animal indicate that adding a superior colliculus lesion to the striate lesion eliminates the residual visual responsiveness in MT in the portion of the visual field representation covered by both lesions. These results suggest that MT, and possibly other extrastriate areas as well, operate at least partially in parallel with striate cortex.

365.1 CYTOARCHITECTURE OF THE OPOSSUM SUBSTANTIA NIGRA PARS LATERALIS. T.P. Ma and J.C. Hazlett. Department of Anatomy, Wayne State University, Detroit, MI 48201.

The pars lateralis (SNl) was examined at the light microscopic level using Nissl, Golgi, Bodian reduced-silver, and HRP-labeled material. In the adult opossum, SNl is approximately 3.5 mm in length and extends from just below the level of the oculomotor nerve to mid-mammillary levels. Caudally this sub-nucleus caps the medial aspect of the dorsolateral edge of the cerebral peduncle. Farther rostrally, SNl expands and covers the lateral third of the peduncle. It terminates as a small scattering of cells over the middle third of this fiber bundle. Throughout most of its course, SNl is separated from the pars reticulata by a cell free zone. Neurons within the SNl can be divided into three groups on the basis of cell size. Small cells (less than 18 um long axis) comprise less than 5 percent of the total population. Medium-large cells (18-35 um long axis) account for about 85%, while large cells (greater than 35 um long axis) comprise the remainder. After injections of HRP into the tectum, striatum, and thalamus, measurements of retrogradely labeled SNl cells suggest that neurons in all three size groups project to these these targets. The somata of SNl neurons vary in shape from fusiform to polygonal and most give rise to several robust dendrites. These usually divide into second order branches within 50 um of the cell body. The secondary dendrites are often quite long and are usually sparsely branched. The primary axis for the dendrites is in the dorsolateral and ventromedial direction. However, a significant number of dendrites penetrate into the underlying cerebral peduncle. A few branches are also seen extending into the overlying tegmentum. The cell bodies and dendrites of well-impregnated neurons exhibit numerous filiform and sessile spines with occassional pedunculated appendages. This latter type is more prominent distally. Although some cells have axon initial segments emerging from the soma, most arise from proximal portions of primary dendrites. Examination of Golgi-impregnated material suggests that there are two types of fibers in SNl. The first type is thin, has varicosities along its length, and usually terminates in a single swelling. The second type is thicker and gives off terminal bulbs. These bulbs are connected to the principal axon by thin stalks and often form flower spray-like terminal arborizations.

365.2 CORRELATIVE LIGHT AND ELECTRON MICROSCOPY OF HRP STAINED NIGRO-STRIATAL NEURONS LOCALIZED IN PARS RETICULATA OF THE RAT SUBSTANTIA NIGRA. I. Grofova*, B.M. Spann* and H. Kita+. Depts. of Anatomy, Michigan State Univ., E. Lansing, MI. 48824 and +Univ. of Tennessee, Memphis, TN. 38163.

Retrograde tracing studies have established that the nigrostriatal tract originates not only in pars compacta(SNC) but also in pars reticulata(SNR) of the substantia nigra. Nigrostriatal neurons are scattered throughout SNR and they are particularly numerous posteromedially. While some of these cells may give rise to non-dopaminergic nigrostriatal connection, it seems that the majority are dopaminergic(van der Kooy,D. et al.,Neuroscience,6:345-357, 1981).

In the course of experiments aiming at physiological identification and intracellular labeling of nigrostriatal neurons, two of the recovered cells were localized in SNR. One cell was antidromically activated from the striatum with a latency longer than 7ms suggesting that it was a dopaminergic neuron. The other cell was not physiologically identified but exhibited similar morphological features and was located in the posteromedial SNR. Axons of both cells were followed into the medial forebrain bundle.

The nigrostriatal SNR neurons were morphologically distinct from previously described SNR projection neurons(Grofova,I. et al., J. comp.Neurol.,208:352-368,1982). While several of the dendrites remained in SNR and made up a large dendritic field similar to that of nigrothalamic and nigrotectal cells, a thick dendritic stem coursed dorsally and contributed together with its varicose branches to the dendritic lattice of SNC. Furthermore, spines were frequent on proximal dendrites and a few were seen on the somata. The most striking difference concerned the axons which were delicate, did not collateralized and exhibited fairly regularly spaced swellings suggesting that they may establish synapses en passant. Electron microscopic examination revealed that the axons were unmyelinated during their intranigral course. Thin axonal portions averaged 0.4um in diameter and contained parallel arrays of microtubules and vesicular organelles resembling portions of SER. They were occasionally found in close apposition with large dendrites. Axonal varicosities measured up to 1.7μm in width and 3.5μm in length and contained numerous mitochondria but no accumulation of synaptic vesicles. The most common type of nigral boutons which contain densely packed large ovoid synaptic vesicles and are generally believed to represent terminals of striatal fibers were occasionally found in synaptic contacts with the distended portions of axon.

These observations offer the first positive ultrastructural identification of nigrostriatal SNR cells and axons and suggest that the axonal varicosities do not form structurally defined synapses but may be postsynaptic to presumed striatal terminals.
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365.3 POSTNATAL ORGANIZATION OF THE NIGROSTRIATAL SYSTEM: ANALYSIS BY RETROGRADE TRANSPORT STUDIES AND FLUORESCENCE ACTIVATED CELL SORTING. J.J. Lopez-Lozano, D.M. Gash, J.F. Leary* and M.F.D. Notter. Departments of Anatomy and Pathology, University of Rochester, Rochester, NY 14642.

The mesostriatal system, its cells of origin and pathways, and in particular the nigrostriatal system, has been described by using anatomical and chemical techniques. However, these previous studies did not provide a complete description of the prenatal development of the system. In particular, in neonatal animals, the morpho-histochemical characteristics and distribution of its neurons, and the projections of its fibers remain partially unknown. The present experiment examines this problem by (1) retrograde tracing studies and by (2) flow cytometry and cell sorting techniques. Long-Evans rats 2-, 5-, and 8-days old received unilateral or bilateral injections into the caudate-putamen with one or two of the following markers: HRP-WGA 1% (0.2-0.6 ul), FITC-Succynil-WGA 1% (0.3-0.6 ul), PI 1% (0.3-0.5 ul), or Doxorubicin 0.5-2.5% (0.3-0.5 ul). The appropriate coordinates were calculated using a stereotaxic atlas of the neonatal animals developed by one of us (J.J.L.L.). Following a survival time of 24 hr the neonatal rats were perfused intracardially with an appropriate fixative. The study of the retrograde labelled cells, with special reference to the ventral mesencephalic region, was made by means of: using a HRP-TMB reaction, immunohistochemistry of WGA (WGA-ICC), and/or by means of fluorescence microscopy. A combination of Tyrosine Hydroxylase immunohistochemistry (TH-ICC) and WGA-ICC was used to examine the existence and percent of the retrogradely double-labelled dopamine cells in several animals. After demonstrating the localization, topographical distribution, and histochemical characteristics of the mesencephalic labelled cells, a second group of animals was bilaterally injected with FITC-SWGA into the caudate-putamen. Then, they were decapitated and the ventral mesencephalon, free of meninges, was quickly dissected out, minced and placed in a cold Ca-Mg free buffer. Tissue was dispersed providing a viability better than 89% and minimum debris. The single cell suspension was later: 1) studied by fluorescence microscopy, TH-ICC, and Neuron-Specific-Enolase-ICC (NSE-ICC); 2) cultured and examined with the cited techniques, and/or; 3) analyzed by Multiparameter Flow Cytometry and cell sorter techniques; and 4) sorted on two parameters according to specific windows determined by multiparameter data analysis as compared to a control group (single suspension of mesencephalic cells with no fluorescence tracer). NSE-ICC and TH-ICC confirmed that the sorted cells were neurons and that a high percent of them contained TH. The pattern of organization of the mesostriatal system including topographic distribution and interhemispheric projections resembled that seen in the adult animal. In addition, the procedures are presently being employed to sort select populations of mesencephalic cells for in vitro and in vivo studies.

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365.4 LOCALIZATION OF GABA IMMUNOREACTIVITY IN MONKEY SUBSTANTIA NIGRA: A LIGHT AND ELECTRON MICROSCOPIC STUDY. G.R. Holstein, T. Pasik, P. Pasik and J. Hamori*. Depts. of Neurology and Anatomy, Mount Sinai Sch. Med., CUNY, New York, N.Y. 10029, and 1st Dept. of Anatomy, Semmelweis Univ. Sch. Med., 1450 Budapest, Hungary.

Histochemical, biochemical, anatomical and electrophysiological studies have suggested the presence of GABA or GAD in substantia nigra (SN), localized primarily in axon terminals, probably originating from Spiny I neostriatal neurons. Other possible sources of GABAminergic input to SN, e.g. from globus pallidus and nucleus accumbens septi, have also been described. To directly demonstrate and characterize the GABAminergic elements present in primate SN, an antiserum of known specificity (Hodgson et al., J. Histochem. Cytochem., 1985), raised against a GABA-BSA conjugate, was utilized in an immunocytochemical procedure. Vibratome 50 μm coronal sections from two adolescent monkeys (1 M. mulatta, 1 M. fascicularis and one 4-week-old monkey (M. mulatta) were incubated in 1 1:1200 dilution of antiserum and further processed with the PAP technique. No reaction product was visible in control sections.

Light microscopy revealed the presence of reaction product in fibers of both pars compacta (pc) and pars reticulata (pr). Small caliber (0.4-2.0 μm dia.) GABAminergic processes traversed the crus cerebri ventrodorsally, entered pr from the ventral aspect, and branched profusely within the nucleus. Immunoreactive fibers were seen surrounding unreacted neuronal somata. Varicosities (1.0-2.1 μm dia.) were apparent along the labeled axons, spaced 1-2μm apart. In pr, single fibers could be followed up to 45 μm through the neuropil. In contrast, the immunostained processes in pc could only be traced over shorter distances; these fibers were finer, more varicose, and formed a less dense plexus. In addition, polygonal or fusiform shaped immunoreactive neurons were observed in both regions.

Using serial section electron microscopy, small immunoreactive boutons with small dark mitochondria and densely packed vesicles (50 nm dia.) were apparent throughout pc and pr. These labeled profiles were abundantly represented, and formed synapses with large and medium size, pale dendritic elements. Such contacts were characterized by symmetrical membrane thickenings with dense projections visible in the presynaptic profile. A series of immunostained boutons could be seen arranged side-by-side, impinging on large pale dendrites visible in longitudinal and cross section.

These observations provide direct positive evidence for the presence and localization of GABAminergic fibers and terminals in monkey SN. Fibers are present in both pr and pc and, based on morphologic criteria, appear to mediate inhibition. The large pale dendritic elements observed postsynaptically may well represent the processes of dopaminergic SN neurons.

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365.5 GLUTAMIC ACID DECARBOXYLASE AND ENKEPHALIN IMMUNOREACTIVE AXONS IN THE NEOSTRIATUM SYNAPSE WITH STRIATONIGRAL NEURONS. N. Aronin, K. Chase* and M. DiFiglia. Depts of Medicine, U. Mass. Med. Ctr. Worcester, MA 01605, and Neurology, Massachusetts General Hospital, Boston MA 02114.

There is evidence that neostriatal glutamic acid decarboxylase (GAD) and immunoreactive enkephalins are located in medium spiny neurons, which have long efferent axons with collaterals that terminate locally. Since numerous GAD and enkephalin-containing axons form synapses in the neostriatum, their synaptic relationship with striatofugal cells seemed likely and was examined in the present study.

Sprague-Dawley rats (n=3) were anesthetized and the substantia nigra was pressure injected with 0.5 µl of 30% WGA-HRP (Sigma). After 20 h the animals were perfused with cold buffered 4% paraformaldehyde/0.1% glutaraldehyde. To identify the retrogradely-labeled cells, tissue sections of caudate were incubated serially with $CoCl_2$ and 3',3'-diaminobenzidine containing 0.01% $H_2O_2$. Sections were immediately processed by the PAP method, using antisera directed to GAD (from J-Y Wu, Hershey, PA) and leu-enkephalin (from E. Webber, Portland, OR). Control sections, in which primary antisera were omitted or the leu-enkephalin antiserum was preabsorbed with leu-enkephalin (50 µg/ml), lacked specific staining. At the light microscopic level regions of the caudate containing a high density of retrogradely-labeled cells (marked by large reaction granules in their cytoplasm) and immunoreactive axon terminals, were selected for electron microscopic study.

At the ultrastructural level retrogradely labeled cells were of medium size with unindented nuclei and exhibited numerous electron dense bodies in their cytoplasm. Dense granules were also present in primary and secondary dendrites. Immunoreactive GAD or enkephalin containing axons terminals were identified by the presence of a dense precipitate of peroxidase reaction product in the axoplasm and on the membranes of vesicles. Many of the immunoreactive GAD and enkephalinergic boutons formed symmetric synapses with retrogradely-labeled somata and dendrites. Frequently in single sections two or three immunoreactive GAD or leu-enkephalin axon terminals synapsed with the same striatofugal projection neuron.

GABA and enkephalins are thought to have inhibitory roles in the basal ganglia and to influence neurons in the nigra directly via axon terminals of striatofugal projection cells. Present findings show that GABA and enkephalins may also affect nigral function indirectly in the neostriatum via monosynaptic contacts with striatonigral neurons. Supported by NIH grants AM-01126 (NA) and NS 16367(MD).

365.6 STRIO-NIGRAL VERSUS INTRINSIC GABA-ERGIC TERMINALS AND THEIR POSTSYNAPTIC PARTNERS IN RAT SUBSTANTIA NIGRA. C.Nitsch*, W.H.Oertel and R.Riesenberg* (SPON: European Neuroscience Association). Anatomical Institute, University of Munich, D-8000 München 2, F.R.Germany.

In an attempt to reveal the synaptic interrelation between GABAergic boutons of various origin and GABAergic neurons in rat substantia nigra (SN) pars reticulata, different forebrain lesions were performed, i.e. ibotenic acid injections in striatum, hemilesion at the level of the globus pallidus and of the frontal pole of SN. After 2 to 7 days survival, vibratome sections of SN were treated with an antiserum against glutamic acid decarboxylase (GAD) as marker for GABAergic elements. Immunoreaction was visualized with the PAP method. The sections were further processed for ultrastructural analysis.

After hemilesion at the level of the globus pallidus, degenerated boutons were found in contact with GAD-immunoreactive nerve cell bodies and their dendrites. In the same area, contacts with non-immunoreactive dendrites were also encountered. On the other hand, an appreciable number of GAD-containing boutons were preserved, in synaptic contact with both, GAD-positive and GAD-negative neuronal elements. After circumscribed lesions in the striatum, the majority of degenerated boutons contacted GAD-immunoreactive postsynaptic elements. Few degenerated boutons were found on GAD-negative dendrites, suggesting that a minority of the striatal input may terminate on dopaminergic projection neurons. However, even after complete prenigral interruption of all forebrain input, numerous GAD-immunoreactive boutons in contact with GAD-positive and GAD-negative neuronal elements were preserved. This result provides strong evidence for the existence of an elaborate GABAergic innervation intrinsic to SN.

It is concluded that the strionigral pathway communicates with the nigrostriatal projection neurons mainly via GABAergic interposal.

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365.7 EVIDENCE FOR TRANSNEURONAL DEGENERATION OF DOPAMINE NEURONS IN THE SUBSTANTIA NIGRA OF THE RAT BY DESTRUCTION OF NEURONS OF CAUDATE NUCLEUS. M. Saji* and D.J. Reis (SPON: S.F. Morrison). Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

We sought to establish if selective destruction of intrinsic neurons of the caudate nucleus (CN), the principal target of dopaminergic (DA) neurons of the substantia nigra pars compacta (SNc) and the source of neurons of the striatonigral tract would affect the integrity of DA neurons of SNc. Ibotenic acid (IBO), an excitotoxin destroying neurons but not fibers of passage, was stereotaxically injected in three sites (4ug/0.4ul) of the head and body of CN in anesthetized rats. At various days, the rats were killed. Sections were stained with antibodies to tyrosine hydroxylase (TH) or thionine and analyzed quantitatively. IBO resulted in a 80-90% loss of neurons in CN by 10d. Associated was complete loss of anterograde transport of HRP-WGA in terminals of the striatonigral tract from CN to SN, pars reticulata (SNr). Within 10 days there was a increase (greater than 200%) in glia in the SNr peaking at 14d at 260% of the unchanged contralateral SNr. Gliosis of the SNc increased very modestly beginning by 21d to maximum (150%) at 30d control. Shrinkage of some DA dendrites in SNr appeared between 5-10d with a progressive reduction in size of DA neurons in SN. The volume of SNr was reduced to 73% by d10 and to 51% between d30-60. Despite atrophy the number of SNc neurons which could still retrogradely transport HRP-WGA injected into CN was unchanged. Between d30-60 16% of the DA neurons in SNc died.

We compared the IBO-initiated changes to those of the retrograde reaction elicited by axotomy of DA neurons produced by microinjection of 6-OHDA into three sites of the CN (4 ug/0.4 ul). Axotomy resulted in a rapid loss of TH immunoreactivity in CN by 75-90% by 7d indicating axonal degeneration. The numbers of CN neurons was unchanged. Seventy percent of DA neurons in SNc began to die by 7d postlesion. By d21 70% of DA neurons were dead. Retrograde transport of HRP-WGA from CN to SNc was almost eliminated but anterograde transport to SNr was unchanged. A modest gliosis was greater in SNc than in SNr (maximal SNc 190 vs. 160% in SNr) between 14 and 21d. The volume of SNr was unchanged until 60d postlesion. We conclude: destruction of neurons in CN by IBO results in rapid dendritic shrinkage, reduction in the volume of SNr and delayed loss of DA neurons in SN associated with marked gliosis in SNr with little in SNc. Such changes differ qualitatively and quantitatively from retrograde cell loss produced by axotomy. It is most probable that the loss of TH neurons represents anterograde transneuronal degeneration of DA neurons produced by loss of striatonigral projection.

365.8 NEUROCHEMICAL CORRELATES OF TRANSNEURONAL CELL LOSS OF DOPAMINERGIC NEURONS OF SUBSTANTIA NIGRA. J.I. Woo*, M. Saji*, S.P. Arneric and D.J. Reis (SPON: A.B. Judd). Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

It has been proposed that destruction of intrinsic neurons of the caudate nucleus (CN) by neurotoxins such as ibotenic acid (IBO) destroys local neurons while preserving innervating fibers of dopamine (DA), neurons of the substantia nigra (SN). We have recently found that such treatment results, after a delay of about 1 m, in progressive shrinkage of dendrites, neuronal atrophy and death of some DA neurons in the SN. Such changes contrast with the rapid and more extensive loss of DA neurons produced by axotomy (Saji et al., this volume), and have been interpreted as reflecting anterograde transneuronal degeneration of DA neurons by lesions of striato-nigral projection. We sought to characterize the associated biochemical changes in CN and SN produced by microinjection of IBO into the CN and to contrast these with changes produced by axotomy elicited by local injection of 6-OHDA into the CN.

The activities of glutamic acid decarboxylase (GAD), choline acetyltransferase (CAT) and tyrosine hydroxylase (TH), markers of GABAergic, cholinergic and dopaminergic neurons, respectively, were measured in CN and SN at various days after lesions. The sodium-dependent uptake of $^3$H-DA into terminals of the CN was also measured. IBO was microinjected into three sites of the head and body of the CN (3.0ug/0.3ul). Groups of 5-8 rats were killed at various days thereafter and CN and SN removed by micropunches. CN. In the ipsilateral CN, IBO produced a rapid reduction to 35% (n=5-8, $p \leq 0.01$) of CAT activity by 48 hrs with a more gradual decline in GAD activity (to 34%, n=5-8, $p < 0.001$) by 8d. TH acitivity increased transiently by 72h and recovered by 4d. However, between 21-50d it progressively declined to 60-75% of control. $^3$H-DA uptake, unchanged at first, gradually declined after d21 reaching 34% of control by d56 (n=5, $p < 0.001$). SN. In ipsilateral SN, GAD activity declined to 56% by d14. TH activity was unchanged.

In comparison, axotomy of DA neurons produced by 6-OHDA differed: CN. By 8d in pisilateral CN, TH was reduced to 15%, CAT increased by 34% and GAD was unchanged ($p < 0.001$). At 4w, TH remained reduced while CAT and GAD returned to control. SN. The enzymes were unchanged by 8d in SN. By 4w, TH activity was reduced to 54% of control.

These results, taken with the morphological data (see Saji et al., this volume) indicate that destruction of intrinsic neurons of the CN by IBO acutely produces rapid loss of intrinsic GABAergic and cholinergic neurons, with the projections of the GABA neurons degenerating at a slower rate, and with preservation of DA terminals in CN. However, after a delay of many weeks, there is a gradual loss of DA terminals in the CN reflecting the delayed atrophy and death of DA neurons of SN. The delayed degeneration differs qualitatively and quantitatively from that of the retrograde reaction and are consistent with an anterograde transneuronal degeneration of DA neurons consequent to loss of innervation from the striato-nigral tract.

365.9 LOCALIZATION OF CALCIUM BINDING PROTEIN IN STRIATONIGRAL AND NIGROSTRIATAL SYSTEMS IN THE RAT AND MONKEY.
C.R. Gerfen, K.G. Baimbridge† and J.J. Miller†, Laboratory of Neurophysiology, NIMH, Bethesda, MD and †Dept. of Physiology, University of British Columbia, Vancouver, BC, Canada.

Two distinct striatonigral projection systems originate from the "patch" and "matrix" compartments of the striatum (Gerfen, Nature 311:461,1984). The striatal patches project to dopaminergic neurons in the substantia nigra whereas the matrix projection is distributed to nondopaminergic nigral neurons. The present study shows that antisera to calcium binding protein (CaBP) isolated from human brain specifically labels the striatonigral neurons in the matrix compartment and is nearly absent in the opiate receptor-rich patches in the brains of both monkeys and rats. Nearly all projection neurons in the ventromedial three-fourths of the striatal matrix are labeled for CaBP, as are fibers and terminals that correspond to the known distribution of striatal efferents. Thus, CaBP marks nearly the entire projection system from the striatal matrix and shows that such nigral afferents are densely distributed in the pars reticulata and avoid areas that contain dopaminergic cells labeled for tyrosine hydroxylase-immunoreactivity (TH). This pattern is particularly striking in the monkey substantia nigra in which there are many areas of the pars reticulata containing dopaminergic cells that show a clear absence of CaBP terminal labeling. The localization of CaBP to the matrix-striatonigral projection system is in marked contrast to the distribution of peptide markers of the patch-striatonigral systems that target nigral dopaminergic cells.

The nigrostriatal system has also been shown to be organized in terms of the striatal compartments (Gerfen, Neurosci. Abstr. 10:9, 1984). Dopaminergic projections from the ventral tegmental area (VTA) selectively innervate the striatal matrix. The subtantia nigra contains mixed types of nigrostriatal neurons, some dopaminergic neurons project to the matrix whereas others project to the patches, and nearly all nondopaminergic nigrostriatal neurons project to the matrix. CaBP is localized to most VTA and to a subset of substantia nigra dopaminergic cells (co-localized with TH) and to a small number of nondopaminergic pars compacta cells. This pattern of distribution and the absence of CaBP in terminals in the striatal patches suggest the preferential distribution of this protein to midbrain cells projecting to the striatal matrix.

In both rat and monkey CaBP labels nearly the entire matrix-striatonigral system and the nigrostriatal system to the matrix. Whereas each of these systems are composed of mixed types of neurons, with individual neurons containing different transmitter(s), CaBP provides a common marker of each entire system, in addition to their connections, which may be related to the functional significance of the compartmental organization of the basal ganglia.

365.10 THE DOPAMINE-CONTAINING INNERVATION OF STRIOSOMES: NIGRAL SUBSYSTEMS AND THEIR STRIATAL CORRESPONDENTS. J. Jimenez-Castellanos* and A.M. Graybiel. Whitaker College, MIT, Cambridge, MA 02139.

Specialized relations between the substantia nigra pars compacta (SNpc) and striosomal compartments of the striatum are suggested by two types of experimental finding. First, the dopamine-containing fibers forming the "dopamine islands" during development innervate striatal compartments that are the forerunners of striosomes (Graybiel'84). Second, striatal projection neurons lying in striosomes are labeled by retrograde tracer injections centered in the medial SN or SNpc (Fishell & van der Kooy,'84; Gerfen'84), whereas there is predominant labeling of extrastriosomal matrix neurons following injections centered either in the pars reticulata of the SN (SNpr)(rat, Gerfen'84) or in the pallidum (Graybiel et al'79). In an effort to determine the origin of the dopamine island fibers, and to compare directly nigrostriatal and striatonigral compartmentalization in the striatum, we labeled nigrostriatal afferent fibers and striatonigral projection neurons in 15 cats by placing bilateral deposits of wheatgerm-horseradish peroxidase agglutinate and/or $^{35}S$ methionine into the SN and adjoining cell groups. Tyrosine hydroxylase (TH)-positive subdivisions in the midbrain were identified by reference to sections stained for TH. Those injected included A8 and, in the SNpc, two zones: (1) a caudal, medial and ventral zone with densely packed TH-positive cells probably including the ventrally-extending TH-positive cell nests farther rostrally and laterally, and (2) a dorsal, rostral and lateral zone with diffusely distributed cells continuing into SN pars lateralis (SNpl). In the caudate nucleus there was preferential labeling of fibers (F) innervating dorsal striosomes with deposits centered in caudal zone 1 of SNpc. A lateral component of striosomal innervation appeared with deposits probably affecting lateral zone 1 as well as lateral zone 2 and SNpl. Labeling of cell bodies (CB) in the striosomal system was ordered. A caudal zone 1 injection labeled CB in dorsal striosomes. More dorsal deposits in medial zone 2/A8 labeled CB in ventral striosomes. A deposit centered in SNpl labeled CB in caudoventral striosomes of the caudate nucleus as well as putamenal CB. Labeling of CB in the matrix occurred with deposits centered in SNpr. CB-poor striosomes were present and, evidently depending on the involvement of SNpc/A8, also CB-rich striosomes.

These findings suggest differential origins for the nigrostriatal innervations of striosomes and matrix. The cell-dense zone 1 of SNpc and probably also A8 appear to innervate striosomes. Zone 2 of SNpc may contribute to the innervation of the matrix. The fact that deposits in zone 1 label both fibers and cell bodies in dorsal striosomes indicates further that the dorsal island system may be part of a nigrostriatonigral loop. Finally, in the cat, as in the rat (Gerfen'84), medial SNpc/A8 (or at least medial SN/A8) receives an input from striosomes, whereas many matrix neurons project to lateral SN, apparently to SNpr. Funded by NSF BNS83-19547.

365.11 BEHAVIORAL SENSITIZATION TO DOPAMINE STIMULANTS INDUCED BY CHRONIC NEUROLEPTIC AND CHRONIC COCAINE TREATMENT: ROLE OF NIGRAL GABA RECEPTORS. S.E. Bachus & K. Gale. Dept. Pharmacology, Georgetown Univ. Sch. Medicine & Dentistry, Washington D.C., 20007.

Stimulation of GABA receptors in substantia nigra elicits stereotyped behaviors resembling those elicited by systemic dopaminergic (DA) stimulants (Scheel-Kruger et al., Neurosci. Lett. 4:351, 1977), and may, in response to striatonigral transmission, mediate DA stimulant-induced stereotypies. Consistent with this idea, chronic blockade of DA receptors by neuroleptic drugs, which enhances behavioral responses to dopamine agonists (Tarsy & Baldessarini, Nature New Biol. 245:262, 1973), also increases GABA receptor binding in nigra (Gale, Nature 283: 569, 1980). Thus, changes in nigral GABA function may contribute to altered response to DA stimulants. Stereotypies are also augmented by repeated exposure to the DA stimulant cocaine (COC). The mechanism of this effect is not well understood, nor is it clear whether it involves the neural changes which mediate neuroleptic-induced sensitization. To approach this question we examined functional changes in nigral GABA transmission following chronic treatment with chlorpromazine (CPZ) or cocaine (COC). As an index of nigral GABA receptor sensitivity, we evaluated the behavioral response to intranigral muscimol.

Adult male Sprague-Dawley rats received either CPZ (20 mg/kg) or vehicle daily for 8 wks, or COC (cumulative dose of 120 mg/kg/day) or vehicle for 6 days. Both drugs caused marked behavioral sensitization to DA stimulants. Three to 5 days after cessation of chronic treatment, rats were tested for behavioral responses to 2.5-5 ng muscimol infused bilaterally into substantia nigra.

Consistent with the results of GABA binding studies, CPZ-treated rats showed a significantly more intense behavioral response to intranigral muscimol than did controls. Gnawing and repetitive restricted head movements and forelimb alternation or flexing were exhibited by these rats following a dose of muscimol which produced only sniffing and locomotion in controls. In contrast, rats exposed to chronic COC did not show enhanced stereotypy in response to intranigral muscimol, despite the fact that they exhibited sensitization to COC-induced stereotypy.

The neural mechanisms responsible for COC-induced sensitization to DA stimulants are therefore dissociable from those underlying neuroleptic-induced DA sensitization. In the latter case, our data indicate an involvement of nigral GABA receptor supersensitivity, which develops secondary to chronic blockade of striatal DA receptors. On the other hand, it appears that changes in nigral outflow are not involved in the sensitization produced by chronic COC. While we have implicated nigral outflow in the expression of COC stereotypies (Bachus & Gale, Soc. Neurosci. Abst. 10: 114, 1984), changes in these behaviors consequent to chronic COC exposure must depend upon alterations in other pathways. Supported by HHS grants DA02206 & NS07453

365.12 THE CO-OCCURRENCE OF SUBSTANCE P-LIKE IMMUNOREACTIVITY AND DYNORPHIN-LIKE IMMUNOREACTIVITY IN STRIATOPALLIDAL AND STRIATONIGRAL PROJECTION NEURONS. A. Reiner. Department of Anatomy and Cell Biology, The University of Michigan, Ann Arbor, MI 48109.

Substance P-like immunoreactivity (SPLI) has been observed in striatopallidal projection neurons and in striatonigral projection neurons in the basal ganglia of all avian, reptilian and mammalian species studied. Recent studies have shown that dynorphin-like immunoreactivity (DLI) is also present in striatopallidal and striatonigral projection neurons in members of each amniote class. The present studies were carried out to determine whether SPLI and DLI are found in two different populations of striatal neurons that have parallel projections or whether they are found in the same striatal neurons.

An immunofluorescence double-labeling procedure (Erichsen et al., Nature, 295, 1982) was used to determine whether SPLI and DLI co-occur in individual striatal neurons and in individual pallidal and nigral fibers in pigeons and turtles. A rat monoclonal antibody against substance P (Accurate Chemical) was used in conjunction with a TRITC-conjugated secondary antiserum (goat anti-rat IgG) to label SPLI-containing neurons and fibers. To label DLI-containing neurons and fibers in SPLI-labeled tissue, a rabbit antiserum specific for the C-terminus of Dynorphin A (1-17) (provided by L. Terenius) was used in conjunction with an FITC-conjugated secondary antiserum (goat anti-rabbit IgG). The dynorphin antiserum was blocked with 100μM leucine-enkephalin to prevent any crossreactive labeling of enkephalinergic neurons.

In colchicine-treated pigeons and turtles, both the medial striatum (the source of projections to the substantia nigra and the ventral tegmental area) and the lateral striatum (the source of projections to the globus pallidus) contained numerous SPLI-containing neurons and numerous DLI-containing neurons. Within the medial striatum, nearly all of these neurons contained both SPLI and DLI. The co-occurrence of SPLI and DLI was also very extensive in the lateral striatum, but not as extensive as in the medial striatum. Although nearly all of the DLI-containing neurons in the lateral striatum were found to contain SPLI, a significant percentage of the SPLI-containing neurons (approximately 10-25%) did not contain observable DLI. Within the globus pallidus, substantia nigra and ventral tegmental area, SPLI and DLI were observed to co-occur extensively in fibers and boutons.

The present results indicate that the co-occurrence of SPLI and DLI in striatopallidal and striatonigral projection neurons in pigeons and turtles is highly extensive. Since striatal organization is generally similar among the members of the various amniote groups, it seems likely that SPLI and DLI co-occur extensively in striatal neurons in all amniotes. This research was supported by NS-19620.

366.1 MATERNAL NACL INTAKE AND OFFSPRINGS' FOOD CHOICES. E. Bird and R.J. Contreras, Dept. of Psychology, Yale Univ., New Haven, CT 06520.

Experimental evidence suggests that the food choices of weanling rats are influenced by maternal diet during gestation and/or lactation. Our interest is in determining whether differences in early dietary NaCl intake modify the salt intake of rats later in life. The present study was designed to assess the influence of maternal dietary salt levels on offsprings' voluntary consumption of salt in the diet at weaning.

Prior to breeding and during gestation and lactation adult female rats were fed a diet either low (.08%), mid (1%), or high (4%) in sodium chloride. Litters were culled to nine pups on postnatal day one. The pups were weaned on day 21 and randomly assigned to one of two experiments assessing voluntary NaCl intake. In one experiment, the rats were fed a sodium-free diet and their elective consumptions of 1.8% saline and water were measured every 24-hr for five days. In the other experiment, salt was presented in food rather than in solution; the rats were given deionized-distilled water to drink and their intakes of no-salt diet and 1.8% NaCl diet were measured every 24-hr for five days.

In the solution choice experiment, there were significant differences between the groups in saline preference, which is defined as saline intake/total fluid intake ($F(2,9)=6.35$, $p<.05$). The mean NaCl preference of the low salt pups (.19) was lower and that of the high salt pups (.42) was higher than that of the mid salt animals (.38). The differences in preference are attributable to significant effects of dietary group on saline intake and water intake ($F(2,9)=4.27$, $p<.05$; $F(2,9)=6.99$, $p<.05$). Compared to the mid salt group, the low salt animals drank less saline and more water and the high salt animals drank more saline and less water. There were no differences between the groups in total fluid intake (saline+water) or food intake.

Preliminary results from the food choice experiment suggest a similar effect; the low salt pups tend to ingest less salt and the high salt pups more than the mid salt group. It is interesting to note that, in the food choice tests, rats from all groups chose a diet whose effective salt level was less than 0.6%. This suggests that, contrary to earlier findings, weanling rats do not choose the maternal diet (or, by extrapolation, its nutrient levels) over alternatives. This observation also calls into question the common practice of maintaining rats on lab chow containing 1% NaCl.

These data indicate that maternal dietary salt levels affect offsprings' elective salt consumption in the diet at weaning.

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366.2 LONG-TERM NEUROCHEMICAL CHANGES AFTER PRENATAL EXPOSURE TO MONOSODIUM GLUTAMATE. B. Frieder and V.E. Grimm*. Center of Neurosciences and Behavioural Research, The Weizmann Institute of Science, 76100 Rehovot, Israel.

Exposure of rats to monosodium glutamate in the mother's diet throughout gestation has been found to result in a later learning deficit and juvenile obesity. The deficit was observed only in a complex black and white discrimination maze where two stimuli were presented simultaneously but not in two other mazes were stimuli were presented separately.

In the present study neurochemical changes resulting from the same prenatal exposure to monosodium glutamate were investigated in the brains of both juvenile and adult rats. Uptake of choline, norepinephrine (NE) and GABA were studied in the cortex and hippocampus of 15, 30 and 60 days old rats. Choline uptake was reduced to 20% at 15 days of age, remained still low (60% of control) at 30 days but was elevated by 32% in the adults. Choline uptake in the hippocampus was also elevated in the adults (by 24%). Norepinephrine uptake in the frontal cortex was reduced by 25% only in adulthood while NE uptake in the hypothalamus was reduced only at 30 days of age. The results indicate that prenatal monosodium glutamate exposure results in long term neurochemical changes. The possible relationship between the behavioural and the neurochemical changes will be discussed.

366.3 THE EFFECT OF PRENATAL ETHANOL EXPOSURE ON INTRAVENOUS ETHANOL SELF-ADMINISTRATION BY THE ADULT BEAGLE. J.M. Johnson*, M.B. Waller*, J.R. Pick* and J.H. Wilson* (SPON: M.J.W. Chang). Div. of Lab. Animal Med., Univ. of North Carolina, Chapel Hill, NC 27514.

Pregnant beagle dogs were intubated twice daily with 1.8 g/kg ethanol (25% w/v in water) throughout gestation. Control bitches received isocaloric sucrose. All bitches received a formulated diet containing 16.5% lactalbumin protein. Ethanol treated animals consumed more food than controls and were therefore pair-fed to controls. Following normal delivery, litters were culled to a maximum of 4. The offspring were weaned at 5 weeks and raised to adulthood. Starting at approximately 20 months of age, permanent indwelling jugular catheters were surgically implanted in offspring from each maternal treatment group (Control = CONT, Ethanol Treated = ETOH). Following recovery from surgery, each animal was placed in a sound attenuated operant chamber and, upon pressing a lever (FR-1), received 0.100 g/kg ethanol (15% w/v in saline) via an infusion pump. When responding stabilized, daily sessions of 2 hr duration were conducted at injection doses ranging from 0.025 to 0.100 g/kg. CONT offspring, at an injection dose of 0.075 g/kg, self-administered ethanol in amounts ranging from 0.8 to 2.9 g/kg/session ($N = 4$) resulting in end-session blood ethanol concentrations (BEC) of 81 to 268 mg/dl. ETOH offspring ($N = 4$) administered ethanol in amounts ranging from 2.9 to 3.4 g/kg/session (BEC: 280-338 mg/dl). Since it was observed that most self-administration responding occurred during the initial 20 min of a 2 hr session and declined rapidly thereafter, a second study was conducted in which representative animals were subjected to sessions of 20 min duration. Total amounts of ethanol administered ranged from 0.8 to 1.6 g/kg (BEC: 110 to 203 mg/dl) for the CONT animals ($N = 3$) and from 2.4 to 2.7 g/kg (BEC: 293 and 294 mg/dl) for the ETOH animals ($N = 2$). Although the number of offspring tested was small, the data suggest increased ethanol self-administration by beagles that were exposed prenatally to ethanol. (This work was supported by N.I.A.A.A. Grant No. 5-R01-AA03123-06)

366.4 THE DEVELOPMENT OF HUMORAL AND CELLULAR IMMUNE COMPONENTS FOLLOWING EXPOSURE TO PRENATAL MATERNAL SOUND STRESS IN RATS. S.K. Sobrian, V.T. Vaughn*, L. Crane* and E.F. Bloch*. Dept. of Pharmacology, Howard Univ. Col. of Med., Washington, D.C. 20059.

Last year, we reported that exposure of pregnant rats to environmental stress (electric foot shock) during the last week of gestation significantly reduced IgG levels in the offspring at birth and 4 weeks of age. This deficiency could be eliminated by exposure of pups to mild or moderate postnatal stress. We now report the effects of prenatal exposure to loud noise on the maturation of both humoral and cellular immune responses in the preweanling offspring.

Ten pregnant Sprague-Dawley rats were exposed daily for 60 min on gestational days 15-21 to an 85dB bell that was programed to ring on a variable interval schedule of 30 times during a 60 min period; the duration of each ring was 5 seconds. As females remained in their individual maternity cages during the stress procedure, handling of the pregnant animal was not necessary; 10 control females were left undisturbed. Females delivered naturally and litters were raised by their biological mothers. Total serum IgG and spleen lymphocyte proliferation following mitogenic stimulation with pokeweed (PWM:20ug), phytohemagglutinin (PHA:20ug) or concanavalin A (ConA:20ug) were determined every 7 days from birth to weaning at postnatal day (PND) 21.

Prenatal maternal sound stress (PSS) significantly shortened the gestational period. Birth weights were unaffected but litter size was larger in the PSS females and significantly more female pups were born to PSS mothers than to controls. However, indices of maternal-pup interaction, i.e., latency to nest build and retrieve pups, nursing and grooming, were not significantly different between the groups. Pinna elevation and the development of several reflexes (i.e., surface righting, cliff avoidance and auditory startle) were delayed in PSS offspring; incisor eruption, eyelid dysjunction and free-full righting were unaffected. These difference were unrelated to nutritional factors as body weights and organ/body weight ratios of spleen, thymus and adrenal glands were not significantly different at PNDs 0,7,14 and 21.

The development of the humoral immune response was unaltered by PSS; IgG levels increased during the first 2 postnatal weeks, peaked at PND 14 and then declined. Despite the similar developmental pattern, IgG was consistently lower in PSS offspring; this difference reached significance at PND 21. In constrast to the decrease in humoral immunity, cellular immunity, as measured by lymphocyte stimulation, was enhanced to the T-and B-cell mitogen, PWM, on PND 0 in PSS offspring. An increased proliferation to the T-cell mitogens, ConA and PHA were observed on PND 7 and PND 21, respectively. These data indicate that prenatal sound stress has a differential effect on components of the immune system; humoral components (i.e., B-cell mediated responses) appear to be suppressed by PSS, while T-mediated cellular responses are enhanced.

366.5 PRENATAL STRESS, EMOTIONALITY AND ASYMMETRY IN NEONATAL TAIL POSTURE. Ester Fride* and Marta Weinstock*. (SPON: European Neuroscience Association). Dept. of Pharmacology, Hebrew University-Hadassah Medical School, Jerusalem, Israel.

It has been suggested that the two hemispheres of the brain process emotional responses differently (Flor-Henry, Integr. Psychiat., 1:46, 1983) and that the influence of early experiences on emotionality is unequally distributed in the two hemispheres (Denenberg et al., Science 201:1150, 1978). Behavioural and neurochemical left-right asymmetries can be produced in rats by neonatal handling, a procedure known to reduce emotionality (Sherman et al., Brain Res., 192:61, 1980; Camp et al., Physiol. Behav. 33: 433, 1984). It is therefore possible that prenatal stress, which has been shown to increase emotionality in the offspring (Fride et al., Life Sci. in press; Fride et al., in preparation), may also alter their behavioural asymmetry. This possibility was investigated by determining tail posture in the newborn rat, which is said to predict asymmetry of dopamine pathways at adulthood (Rosen et al., Brain Res., 297: 305, 1984).

We exposed 30 pregnant rats to 3 randomly distributed (unpredictable) sessions of 4 hours of noise (95 db SPL) and flashing lights (20 fc) each week throughout gestation. Thirty pregnant controls were left undisturbed. Mothers were removed from their litters for 3 min. and tail position of the pups was scored for left-, right- or no-preference, within 24 hrs after birth (day 0) and on days 1 and 2.

On day 0, there was no side preference in control rats, whereas prenatally stressed pups showed a shift to the right ($X^2$= 11,3; p<0.001). There were no sex differences on this day. On day 1, controls had developed a leftward bias (p<0.001) which was significant only for males. By day 2, the female control pups had also developed a leftward bias (p<0.1), while the prenatally stressed pups still showed a deviation to the right, although somewhat reduced (p<0.01).

These results demonstrate that unpredictable prenatal stress causes a shift in neonatal tail posture, which may be indicative of asymmetry at adulthood. We suggest therefore, that a prenatally induced change in brain lateralisation may underly our previous findings that mature animals which had experienced prenatal stress, showed an increase in emotionality and a reduction in the ability to cope with stressful situations.

366.6 ONTOGENY OF SEIZURE INCIDENCE, LATENCY AND SEVERITY IN GENETICALLY EPILEPSY PRONE RATS. D.L. Amend, J.E. Franck* and D.L. Hjeresen*. Departments of Psychology and Neurological Surgery, University of Washington, Seattle, WA 98195.

The Genetically Epilepsy Prone Rat (GEPR) has been extensively studied as an animal model of epileptiform disorders (Jobe and Laird, Biochem. Pharm. 30:3137-3144,1981). When tested as adults the GEPR is susceptible to audiogenic, visual, chemical and temperature induced seizures. However, the possibility that seizure susceptibility may be the result of developmenal changes in the central nervous system has not been addressed. The results of the present study indicate that GEPR pups are insensitive to audiogenically-induced seizures through 27 days of age and that seizure incidence, latency and severity increase significantly when rats are retested at 60 days of age.

Eighty GEPR pups from 8 different dams were tested for incidence, latency and severity of an audiogenically induced seizure at 6,9,12,15,18,21,24,27,28,29 or 30 days of age (one test per pup). Seizure testing was conducted in an enclosed cylindrical metal container (40.6 cm dia. X 50.8 cm deep) equipped with an alarm bell with a measured output of 110 db(a). Pups were placed in the chamber and the bell was activated for 60 s or until the onset of a seizure. Seizure severity was quantified on the 9 pt. scale of Jobe, et al. (J. Pharmacol. Exp. Ther. 184:1-10,1973a). At 60 days of age all pups were retested. Results were evaluated by analyses-of variance and by a paired-T test between the initial and 60 day seizure test. Results indicate that sensitivity to audiogenic seizures begins at day 27 post-partum. Prior to Day 27 a brief running fit was noted in a single 18 day old rat. Seizure incidence, latency and severity were significantly greater on days 27, 28, 29 and 30 than all previous days (F's=2.17, 2.21, and 4.71 respectively). When retested at Day 60, seizure incidence, latency and severity were significantly greater than on initial tests regardless of of whether rats had seized during their initial test or not.

The results suggest that seizure sensitivity in the GEPR rat occurs as the result of developmental events in the CNS occuring on or shortly after Day 27 post-partum. These behavioral results are paralleled by the additional finding from our laboratories that benzodiazepine binding in the medial geniculate nucleus declines with age in the GEPR. This suggests that inhibitory mechanisms in the GEPR may decline with age.

366.7 INCREASED CELL DENSITY OCCURS IN THE INFERIOR COLLICULUS OF THE YOUNG GENETICALLY EPILEPSY PRONE RAT. R. C. Roberts*, H. L. Kim* and C. E. Ribak (SPON: D. D. Williams). Dept. of Anatomy, Univ. of Calif., Irvine, Irvine, CA 92717.

Previous work from our laboratory has shown an increase in the number of total neurons as determined by counts of Nissl stained cells and an increase in the number of GABAergic neurons in the inferior colliculus (IC) of the genetically epilepsy prone rat (GEPR) as compared to Sprague-Dawley rats. To determine whether this increase in cell number was present in young GEPRs prior to seizures the brains of GEPRs and Sprague-Dawley rats, 4-10 days of age, were studied. The brains were embedded in paraffin, sectioned in the coronal plane at a thickness of 10 um and stained with cresyl violet. A representative 62,500 $um^2$ grid in the ventral lateral portion of the central nucleus was examined from eight to ten sections across the rostrocaudal extent of the IC; neurons were classified as small, medium and large and counted. In addition, the area of the IC in these sections was determined by a digitizing morphometry computer program.

A statistically significant increase occurred in the numbers of small neurons in the IC of the young GEPR. At 4 days of age there were 116±24 small ,30 ±14 medium and 1±1 large neurons per analyzed grid in the SD rat. In contrast, age matched GEPRs displayed 180±50 small, 18±14 medium and 1±0.3 large neurons in the same area in the GEPR. This is a 55% increase in the numbers of small neurons in the GEPR as compared to the SD rat. At 10 days of age there were 67±22 small, 57±31 medium and 2.5±2.8 large neurons in the SD rat. In contrast, there were 138±48 small, 60±21 medium and 5±5 large neurons in the GEPR. This represents a 105% increase in the numbers of small neurons in the GEPR as compared to the SD rat. However, the numbers of the medium and large neurons were similar in both groups of rats and no significant difference occurred between the areas of the IC.

These data suggest that there is a developmental defect in the generation of small size neurons resulting in an increase in the number of this size neuron in the GEPR IC that is present prior to the seizure state. Since there are similar numbers of medium sized neurons in the 10 day old rats but an increase in numbers of medium sized neurons in the adult, there may be a developmental lag in the growth of this size neuron in the GEPR.

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366.8 EFFECTS OF EXPERIMENTAL HYDROCEPHALUS ON THE MORPHOLOGY OF CORTICAL NEURONS. J.P.McAllister II, T.A.Maugans*, M.V.Shah*, A. Norelle* and R.C.Truex, Jr*. Depts. of Anatomy and Neurosurgery, Temple University School of Medicine, Philadelphia, PA 19140.

Obstructive hydrocephalus produces gliosis and damage to periventricular white matter in the cerebral cortex, and results in severe neurological deficits. However, the changes in neuronal morphology that accompany this disorder are relatively unknown. Recently, we utilized an animal model of hydrocephalus to characterize the dendritic morphology of cortical pyramidal neurons from the most severely affected rats (McAllister,J.P. et al., J. Neurosurg., in press). The present report describes both the cytological and dendritic changes observed throughout the sequence of this disorder. Newborn rats received kaolin injections in the 4th ventricle. These animals were sacrificed at 3,6,7,10 and 12 days post-injection and their brains processed by Nissl and Rapid Golgi methods. Since the parieto-occipital region was the most severely affected portion of the cortex, well impregnated neurons from this area were selected for microscopic analysis and compared to age-matched litter-mate controls. Grossly, ventriculomegaly and cortical thinning were not evident at 3 days post-kaolin injection, but were at 6-7 and 10-12 days. Cellular changes were first seen in experimental animals at 3 days and became progressively more severe. Nissl preparations revealed intense gliosis in the external capsule and absence of neuropil by 10-12 days. Dark shrunken neurons and gliosis were also evident in deep portions of layer V, but not in any other laminae. Golgi impregnation demonstrates that during normal postnatal development, dendrites of pyramidal neurons lose their bead-like swellings (varicosities) and produce dendritic spines. In all cortical layers, pyramidal neurons exhibited progressive increases in dendritic varicosities and decreases in dendritic spines. These changes were variable, however, such that some dendritic segments were smooth with normal complements of spines. Spine reduction and varicosity formation were most notable on distal portions of individual branches and within regions of the dendritic tree closest to the ventricular and meningeal surfaces. Furthermore, the basal dendrites of pyramidal neurons had fewer branches and those that remained were shorter. We conclude that experimental hydrocephalus (1) alters the morphology of cortical neurons and (2) dendritic changes precede gross tissue changes. The dendritic alterations are consistent with other reports of dendritic changes that accompany aging, mental retardation and alcohol exposure. Thus, our observations suggest that hydrocephalus causes deterioration or retardation of somatic and dendritic maturation. The fact that neuronal morphology was not more severely affected may indicate that these effects are reversible. (Supported by BRSG #S07RR05417)

367.1 **EVIDENCE THAT A NERVE INJURY INDUCED, 37K PROTEIN IS A FORM OF APOLIPOPROTEIN E.** M.J. Ignatius, P.J. Gebicke-Harter*, J. Shilling* J.H.P. Skene and E.M. Shooter. Dept. of Neurobiology, Stanford Univ. Med Center, Stanford, CA. 94305.

Upon injury of both peripheral and central nervous system tissue, the synthesis and secretion of a 37K protein is markedly increased (Skene and Shooter, PNAS 80:4169, 1983). This soluble, slightly acidic protein has been shown by a variety of studies to correlate with events of nerve axon growth during development and regeneration. In this report we present evidence that this protein bears striking homology to a well studied serum protein, apolipoprotein E, that is known to be involved in both transport and metabolism of lipids and cholesterol.

In a previous abstract we described the purification of the 37K protein and subsequent production of mono-specific rabbit antisera. In the present study, using this antisera, we examined the cellular origin of the 37K protein in enriched cultures of new born rat oligodendroglia and astroglia. After 7 days in culture the most prominent staining was confined to a population of small (10um) cells, which were generally associated with cellular debris and which contained large inclusion bodies. That these are macrophages or a form of microglia seems likely. Other lines of evidence suggest that glia, rather than these small round cells, are the primary source of the 37K protein in vivo. However, our evidence for 37K protein in macrophage-like cells in vitro raised the possibility that the 37K protein might be similiar to a 35K glycoprotein, apolipoprotein E, that has been shown to be secreted by rat macrophages.

By two criteria, immuno-cross reactivity and amino acid sequence we tested the degree of homology between these two proteins. First in immuno-blots, comparing cross reactivity of both anti-apo E and anti-37K for the two proteins, we found that both antisera recognized each of the denatured proteins. Secondly, we derived a partial amino acid sequence of the 37K protein, for comparison to the complete sequence of rat apo E. Two fragments from the 37K protein of 12 amino acids each, one from the N-terminus and one from an internal region were found to match at all but one position with the known sequence of similiar regions of rat apo E.

The homology of the nerve sheath derived 37K protein to serum apo E suggests that the 37K protein may be involved in similiar events ascribed to apo E, for example in lipid transport and metabolism, yet confined to the nervous system. The presence of the 37K protein during development and its induction after injury indicates that it may play a role in both assembly and maintenance of myelin or axonal membranes during development and regeneration via the mobilization of lipid rich precusors, or in degenerative events whereby lipid rich debris is absorbed and cleared from the site of injury.

This work was supported by NIH grants (MH 17047 and NS 04270), the Isabella Niemala Estate and the Max Planck Society.

367.2 SUBSTANCES ORIGINATING FROM GROWING NERVES INDUCE MORPHOLOGICAL RESPONSES CHARACTERISTIC OF REGENERATION IN INJURED OPTIC NERVE OF ADULT RABBIT. V. Lavie*, S. Sharma, A. Harel*, D. Lobel*, A. Solomon*, A. Doron*, M. Belkin* and M. Schwartz. Dept. of Neurobiology, The Weizmann Institute of Science, Rehovot, Israel.

We have demonstrated that the adult rabbit visual system can express regeneration-associated characteristics after injury if subjected to appropriate growth-associated triggering factors (GATFs). Active GATFs are found in media conditioned by regenerating fish optic nerves or by optic nerves of neonatal rabbit (Schwartz et al., Science, 228:600, 1985; Hadani et al., Proc. Natl. Acad. Sci. USA 81:7965, 1984). The implantation-induced response was manifested by a general increase in protein synthesis in the retina, changes in synthesis of specific polypeptides, and sprouting of new fibers in culture.

The present work provides evidence for the morphological changes following implantation of GATFs in adult rabbits. Axonal fibers and corresponding retinal ganglion cells were retrogradely labelled with horseradish peroxidase (HRP) at various times after optic nerve crush and GATF implantation. Forty-eight hours following HRP application, flat-mounted retinae were processed using DAB as a chromagen. One week following optic nerve injury, light microscopic analysis revealed labelled fibers and ganglion cells in both the implanted and control (injured only or injured and implanted with silicone tubes coated with collagen but free of GATFs) preparations. In control preparations, no staining of fibers or ganglion cells could be detected. In contrast, two weeks or one month after the injury, the GATF implanted preparation showed a high degree of viable ganglion cells with dendritic arborizations and abundant stained fibers, some with growth cones.

Electron microscopic analysis revealed unmyelinated nerve fibers with growth cones embedded in the scaffold formed by glial cells only in the implanted injured nerve.

These results emphasize that GATFs implanted around the injured optic nerve of an adult rabbit induce a morphological response in addition to the biochemical response previously reported. Whether this response leads to complete anatomical recovery or only to abortive growth warrants further investigation.

367.3 MOLECULAR ASPECTS OF OPTIC NERVE REGENERATION IN REGENERATIVE AND NON-REGENERATIVE SYSTEMS. C. Stein-Izsak, A. Harel*, A. Solomon*, D. Neumann*, M. Belkin*, M. Rubinstein * and M. Schwartz. Dept. of Neurobiology, The Weizmann Institute of Science, Rehovot, Israel, 76100.

The induction of regeneration-associated changes following neuronal injury to a non-regenerative adult mammalian CNS has been achieved in our laboratory (Schwartz et al., Science, in press). Application of growth associated triggering factors (GATFs), from non-neuronal cells of regenerating or growing nerves, as an implant around the injured optic nerve of an adult rabbit resulted in a general increase in retinal protein synthesis, changes in synthesis of specific polypeptides and sprouting of new fibers from the retina in vitro (Hadani et al., Proc. Natl. Acad. Sci. USA. 81: 7965, 1984). These changes are characteristic of regeneration in goldfish and other lower vertebrate CNS having a high regenerative capacity. To gain an understanding of the mechanism underlying the action of the GATFs and the resultant changes in the retina, mRNA translation products from retinas of goldfish and rabbit were analyzed under various experimental conditions. The purification and characterization of GATFs from non-neuronal cells of regenerating fish optic nerves was also undertaken.

Poly $(A)^+$ RNA was extracted from retinas of goldfish 2, 4, 8 and 35 days following crush injury to the optic nerve. The contralateral uninjured retina served as control. The mRNA was translated in a reticulocyte lysate cell free system and the translated polypeptides were examined by 2-dimensional gel electrophoresis. Translation products from retinas of regenerating goldfish optic nerves included polypeptides barely detectable in the controls. Among these polypeptides, those of apparent molecular weight 24-28 kDa; 43-49 kDa; 60 kDa; and 65 kDa may be analogous to the growth associated polypeptides described in other regenerative or developing systems. The induction of regeneration-associated characteristics in the retina of the injured rabbit optic nerve implanted with GATFs was reflected by changes in mRNA translation products of molecular weight 16-18 kDa; 28 kDa; 32-35 kDa; 43-47 kDa; 56-60 kDa and some higher molecular weight species.

Thus, in visual systems of both the goldfish (representing a regenerative CNS) and the rabbit (representing a non-regenerative mammalian CNS), regenerative responses in the retina are associated with alterations in mRNA expression.

To isolate and identify the molecule(s) which trigger the regenerative response in the rabbit retina, GATFs from non-neuronal cells of regenerating fish optic nerves were fractionated by Sephadex G-25 chromatography. The active moiety, as assessed by bioassay, resided in a peak of molecular weight less than 5 kDa. This fraction, when further purified by reverse-phase HPLC, yielded several peaks, only one of which was active.The apparent molecular weight of this peak is 3 kDa. We have previously shown that non-neuronal cells of regenerating fish optic nerves display changes in their pattern of pulse-labelled polypeptides and mRNA translation products (Rachailovich et al., Proc. 14th Meet. Soc. Neurosci. 10: 1031, 1984). In addition, GATFs derived from these cells have greater biological activity than GATFs from control nerves. The amino acid sequence of the purified GATF will be used to study the regulation of its expression in regenerating and non-regenerating CNS.

367.4 EXPRESSION OF SPECIFIC NON-NEURONAL PROTEINS DEFINES DISTINCT "GROWTH-STATES" OF REGENERATING AND NON-REGENERATING NERVES IN MAMMALS. H.W.Müller, M.J.Ignatius, P.Gebicke-Härter*, D.H.Hangen* and E.M.Shooter. Dept. of Neurology (H.W.M.), University of Düsseldorf, F.R.Germany and Dept. of Neurobiology, Stanford Univ. Med. Sch. Stanford, CA 94305.

Protein synthesis in non-neuronal (sheath) cells of injured as well as intact mature and developing sciatic nerves and optic nerves from rat was investigated by incubating segments of nerve with ($^{35}S$)-methionine in vitro. The composition of radioactively labeled proteins released into the incubation medium was analyzed by two-dimensional gel electrophoresis and fluorography.

The expression of 4 proteins in sciatic nerve with the apparent molecular masses of 70kDa, 54kDa, 51kDa and 37kDa was of particular interest because of the correlation of their synthesis and secretion with nerve growth and regeneration. The 37kDa protein was synthesized at a significant rate during neonatal development as well as during Wallerian degeneration and axon regeneration following a crush lesion but not in the intact mature nerve. The synthesis of the 37kDa protein was inhibited when proper innervation was established. In contrast, the synthesis remained at almost maximal rates following permanent denervation. The 70kDa protein was synthesized at a significant rate only in response to axotomy thus confining its role to some aspect(s) of nerve repair. On the other hand, the 54kDa and 51kDa proteins were expressed in the intact mature nerve sheath. Their synthesis and release was rapidly inhibited upon axotomy but returned to normal or higher levels within 3-4 weeks post-injury suggesting a function in the maintenance of the integrity of the mature (non-growing) sciatic nerve. Particular growth states (i.e. developing, intact mature, regenerating, permanently denervated) of rat sciatic nerve can thus be identified by the differential expression of specific proteins in non-neuronal (sheath) cells.

A protein which is homologous to the 37kDa protein in peripheral nerve was synthesized at significantly higher rates in optic nerve of neonatal rats than in adult animals. As a specific response to denervation the synthesis of this protein was stimulated approximately 100-fold in the mature optic nerve. However, in contrast to sciatic nerve, the 37kDa protein did not accumulate in the optic nerve. The differences in accumulation of the 37kDa protein correlate with the apparent differences in the ability of peripheral and central axons to regenerate.

367.5 **SEM VISUALIZATION OF EARLY EVENTS IN PNS NERVE REGENERATION WITHIN A TUBULAR PROSTHESIS: EFFECTS OF DIFFERENT PROTEIN ADDITIVES.** L. Kljavin-, R. Madison, C. F. Da Silva, and R. L. Sidman. Departments of Neuropathology and Neuroscience, Harvard Medical School and Children's Hospital, Boston, Ma. 02115.

The present experiments were undertaken to gain insight into the 3-dimensional aspects of the early events of nerve regeneration within tubular prostheses. Twenty adult male C57BL/6J mice received sciatic nerve transections at mid-thigh level and both proximal and distal nerve stumps were sutured into a 6 mm long polyethylene tube, ID 0.76 mm (Clay Adams) to give a final nerve gap distance of 4 mm. The tubes contained either a laminin-containing gel (gift of Drs. Kleinman and Martin, NIH) or a collagen matrix (Vitrogen, Flow laboratories) at the time of implantation. Two animals from each group were sacrificed at 1, 2, 4, 7, 11, or 15 days after surgery, and the tissue cable within the tube processed for SEM analysis.

At day 1 and 2 the laminin gel appears very globular with some smaller collagen fibers, probably Type IV, apparent. There is no overall orientation to either the collagen or the globular masses within the cable. Many presumptive macrophages and red blood cells are present within and on the surface of the cable. By day five the cable is markedly less globular in appearance and more collagen is seen around the periphery of the cable while large channel like openings are seen within the cable itself, suggesting a reworking of the matrix components into a more longitudinally oriented arrangement. By day 7 many more channel like openings are seen in the cable, and a distinct epineurium with much collagen surrounds the boundaries of the cable. Fewer cells are evident within the cable at this time point, and some presumptive unmyelinated axons can be seen. By 11 and 15 days, the cable is begining to look more normal with a distinct epineurium, bundles of unmyelinated axons can be seen surrounded by perineurium, and individual myelinated axons can be seen.

The appearance of the collagen filled tubes is quite different. At days 1 and 2 there is only collagen present in the cable arranged in a random fashion. Some presumptive fibroblasts can be seen migrating on the surface of the cable. By day five the collagen is begining to appear less random and many more presumptive fibroblasts are seen on the surface of the cable and some within the cable itself. By days 7-15 the collagen becomes much more ordered into longitudinal arrays, macrophages are seen, and at the later time point presumptive unmyelinated and a few potentially myelinated axons can be distinguished.

The remaining 8 animals were sacrificed at two weeks for retrograde cell labeling and these animals also suggest a more rapid growth of axons across the nerve gap in the group that received the laminin-containing gel. Animals in this group displayed more labeled sensory and motor cells and more myelinated axons compared to the group that received collagen in the inside of the tube (see also, Da Silva et. al.; and Madison et. al., this volume.) Supported by NIH grants EY05317 (RM), NS20820, NS20822, HD18655 (RLS).

367.6 **QUANTITATIVE EFFECTS OF A LAMININ-CONTAINING GEL, COLLAGEN, OR EMPTY POLYETHYLENE TUBE ON PERIPHERAL NERVE REGENERATION IN VIVO.** C. F. Da Silva, R. Madison, D. Greatorex*, P. Dikkes*, & R.L. Sidman. Departments of Neuroscience, Children's Hospital & Neuropathology, Harvard Medical School, Boston, Ma. 02115

We recently reported an in vivo model to quantify the number of primary motor and sensory cells that send an axon across a 4 mm nerve gap in the mouse sciatic nerve using entubulation repair (Da Silva et. al., Brain Res, in press). **The present studies utilized this model system of PNS regeneration to quantify the effects of different protein additives to the lumen of the tube on the rate and extent of axonal regeneration.**

The sciatic nerve of adult C57BL/6J mice was transected at mid-thigh and proximal and distal stumps were sutured into a polyethylene tube (PT) 6 mm long (0.76 mm I.D.), to give a final gap distance of 4 mm. Animals were divided into three groups of 16 animals each. The first group received an implantation of a PT alone, the second group a PT filled with a laminin-enriched gel (Kleiman, H.K. et al, Biochem.,21:6188-6193, 1982) and the third group a PT filled with a purified preparation of collagen (vitrogen, Flow Labs. Inc.). Four animals in each group were sacrificed following survival times of 2, 4, 6 and 12 weeks, and the distal nerve stump was cut and exposed to an HRP solution. After an additional three days, animals were perfused with fixative. The L3-L5 dorsal root ganglia (DRG) and the lumbar spinal cord were processed for HRP-TMB histochemistry; labelled cells were counted in 40 um serial sections. The polyethylene tubes with the regenerating nerve cables were processed for plastic embedding; the number of myelinated axons in 1 um sections at the mid-point of the cables was determined with a computer-controlled system. The values (mean±SEM) obtained are (*=significant differences between groups):

| | 2 Wk | 4 Wk | 6 Wk | 12 Wk | |
|---|---|---|---|---|---|
| SP.CORD | 494±72 | 713±99 | 713±82 | 587±70* | EMPTY |
| DRG | 803±234 | 1262±168 | 1120±226 | 1707±139 | TUBE |
| MYEL.AX. | 231±121* | 1318±375 | 1201±216* | 1681±218* | |
| SP.CORD | 657±58* | 768±38 | 780±53 | 882±29* | |
| DRG | 1687±311* | 1380±123 | 1634±171 | 1758±167 | LAMININ |
| MYEL.AX. | 1392±483* | 1640±152 | 2031±297* | 2332±91* | GEL |
| SP.CORD | 327±66* | 597±106 | 649±71 | 650±107 | VITROGEN |
| DRG | 322±129* | 1193±128 | 1463±188 | 1589±321 | |
| MYEL.AX. | 100±99* | 1185±163 | 1813±135 | 2176±245 | |

**These results suggest that specific protein additives have differential effects on the rate and extent of PNS regeneration in the mouse, and demonstrate the usefulness of this model system to test manipulations of the microenvironment of regenerating axons.** Supported by NIH grants EY05317 (RM), NS20820, NS20822, HD18655 (RLS).

367.7 **MODIFICATION OF THE MICROENVIRONMENT ALLOWS AXONAL REGENERATION ACROSS A 20 mm NERVE GAP USING ENTUBULATION REPAIR.** R. Madison, C.F. Da Silva., and P. Dikkes*. Departments of Neuropathology and Neuroscience, Harvard Medical School and Children's Hospital, Boston, Ma. 02115

We have recently developed a model to quantify the number of primary motor and sensory neurons that send axons across a nerve gap (Da Silva et. al., Brain Res., in press), and have shown that axonal regeneration is significantly enhanced if a laminin-containing gel is added to the inside of the tube at the time of implantation (Madison et. al., Exp. Neurol.; 88 (3), 767-772, 1985). The present experiments were undertaken to determine if similar modifications to the internal environment of the tube would increase the maximum distance that PNS axons will elongate within a tubular prosthesis. Two different laboratories have reported that the sciatic nerve of rats will not regenerate a nerve cable within a tubular environment if the nerve gap distance is more than 10 mm in length (Seckel et. al., J. Plast. Reconstr. Surg.; 74, 173-181; Lundborg et. al., Exp. Neurol. 76, 361-375).

Eight adult male Sprague-Dawley rats received sciatic nerve transections at mid-thigh level and both nerve stumps sutured into a silicone tube (Cole Parmer, ID-1/16 inch) to bridge a nerve gap of either 15 or 20 mm. The tube was either left empty (N=2), filled with a laminin-containing gel (N=3, gift of Drs. Kleinman and Martin, NIH), or a collagen matrix ( N=3, Vitrogen, Flow Labs). After 4-12 weeks, the distal stump was resectioned 5 mm beyond the end of the tube and sealed into a polyethylene tube filled with an HRP solution. Three days later animals were perfused with fixative and the L3-L5 dorsal root ganglia and lumbar enlargement of the spinal cord processed for HRP histochemistry using TMB as the substrate. The regenerated nerve cable was processed for plastic embedding. All of the animals with laminin-gel(N=2 at 4 wks., 15 mm gap; N=1 at 5 wks., 20 mm gap) or collagen (N=3 at 6-12 wks., 20 mm gap) added to the interior of the tube displayed labeled cells in both the DRG and spinal cord, and a tissue cable connecting proximal and distal nerve stumps. Myelinated axons could be seen in the light microscope to extend as far as 10 mm into the tube. Electron microscopic analysis revealed the tissue cable to be composed of fibroblasts, schwann cells, macrophages, abundant collagen, many unmyelinated axons and numerous small myelinated axons. Neither of the two control animals (empty tube) displayed either a nerve cable in the tube or labeled cells in the DRG and spinal cord when sacrificed at 12 weeks.

**These results suggest that the maximum distance that PNS axons will elongate within a tubular prosthesis can be dramatically increased, at least by a factor of two, by additives to the interior of tube.** Supported by NIH grant EY05317(RM).

367.8 NERVE REGENERATION THROUGH HOLEY SILICONE TUBES. R.E. Coggeshall and C.-B. Jenq. Marine Biomedical Institute and Depts. of Anatomy and of Physiology & Biophysics, University of Texas Medical Branch, Galveston, TX 77550.

Recently a number of laboratories have studied various parameters of peripheral nerve regeneration after the nerve is transected and the stumps placed in an impermeable silicone tube. A reasonable hypothesis is that the tube, being impermeable, is beneficial because it holds various growth factors in a pool around the axons that regenerate from the proximal stump. One way to test this hypothesis is to drill macroscopic holes in the tube so that the general extracellular fluid has free access to the regrowing axons.

To do this, we removed an 8mm segment of adult rat sciatic nerve and the resulting stumps were placed in a silicone tube which had 2 rectangular holes, (0.6 x 25 mm) in its walls. Axons were counted in the distal stump, in the sural nerve and in the medial gastrocnemius nerve 8 weeks following surgery.

The normal rat sciatic nerve contains approximately 8,000 myelinated and 15,000 unmyelinated axons, the normal sural nerve contains approximately 1,000 myelinated and 3,500 unmyelinated axons and the normal nerve to the medial gastrocnemius muscle contains approximately 300 myelinated and 450 unmyelinated axons. After the above surgery, the distal stump for sciatic nerve contains approximately 8,000 myelinated and 16,000 unmyelinated axons, the sural nerve contains approximately 800 myelinated and 2,000 unmyelinated axons and the nerve to the medial gastrocnemius muscle contains approximately 250 myelinated and 500 unmyelinated axons. These numbers are in general closer to the normal numbers than after similar regeneration through an impermeable tube. Controls assured that not enough axons entered or left the holes in the tube to influence the counts. Thus, if a silicone tube is made permeable by putting large holes in its wall, the regeneration that results may be superior at least in terms of the parameters measured in the present study.

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367.9 SCIATIC NERVE, MUSCLE OR COLLAGEN MATRIX FOR TRANSECTED SPINAL CORD REPAIR. J.C. de la Torre, M.T. Richard*, and L.P. Ivan*. (SPON: J. Metuzals). Division of Neurosurgery, Univ. of Ottawa, Health Sciences and Ottawa General Hospital, Ottawa, Ont. K1H 8M5.

One the critical problems in mammalian spinal cord reconstruction following a severe injury that results in permanent paralysis, is choosing a material to replace the defect produced by the lesion. In our continuing attempt to collate the optimum factors that may promote regeneration of severed neuronal processes, we examined tissue matrices composed of autologous sciatic nerve, skeletal muscle and cell-free bovine collagen and used these to bridge the transected gap in rat spinal cord. Rats were divided into 4 groups: sciatic nerve implant (N), muscle implant (M), collagen implant (C) and gelfoam controls (G). All rats underwent a 200 gram/centimeter force dorsal cord injury at $T_{10}$. Rats were allowed to recover for 14 days then were transected at $T_{10}$ and 2-2.5 mm of cord tissue was removed by trimming the proximal-distal cord stumps using a Codman ML-10 $CO_2$ laser. Each group received their respective implant which completely filled the space left by the laser trimmed proximal-distal cord stumps.

After 2 weeks, rats underwent somatosensory evoked potentials (SEP) and neurological evaluation. Rats were killed for light microscopic examination of cord tissue using conventional stains and SPG histofluorescence for catecholamines. Results indicate that the interfaces of proximal cord tissue with C and to a lesser extent M, contained catecholamine varicosities when examined 15 days following implantation of these bridge matrices. There were no catecholamine varicosities visible in the proximal cord tissue interface with S or G matrices. The intraaxonal catecholaminergic density in the proximal host tissue near the lesion was the same in all groups. Only 1 rat in group C showed catecholamine varicosities in the distal cord after 2 weeks. At the time of sacrifice, all SEP's and the neurological evaluation were negative for all rats.

We conclude from this data that the cell-free collagen matrix implant is superior to muscle, sciatic nerve and gelfoam bridges with respect to supporting axonal catecholaminergic proliferation ostensibly growing from proximal host tissue regeneration.

367.10 EXOGENOUS MATRIX PRECURSORS STIMULATE THE TEMPORAL PROGRESS IN A SILICONE NERVE REGENERATION CHAMBER. L.R. Williams and S. Varon (Spon: E. Keithley). Dept. of Biology, Sch. of Med., Univ. Calif. San Diego, La Jolla, CA 92093.

Successful peripheral axonal regeneration across a 10 mm gap within a silicone chamber is dependent on at least two sequential events: 1) a fibrin matrix must form and connect the proximal and distal nerve stumps; and 2) nonneuronal cells that possess properties supportive of axonal elongation must migrate into and replace the matrix (Williams and Varon, J. Comp. Neurol., 1985). When chambers are implanted empty or prefilled with saline, cell migration and axonal regeneration are delayed by the 5-7 days required for formation of a complete matrix bridge in situ. We now report that the temporal progress of cell migration and axonal regeneration can be stimulated by prefilling the chamber with a solution of fibrin matrix precursors. Proximal and distal stumps of a transected rat sciatic nerve were sutured into the ends of a silicone chamber (1.8 mm I.D.) leaving a 10 mm gap between the stumps. Control chambers were prefilled with phosphate-buffered saline (PBS). Experimental chambers were prefilled with a solution of citrated rat plasma obtained via cardiac puncture that was dialyzed against PBS to remove calcium-citrate chelates (dialyzed plasma, DP). Fibrinogen (Fg) content was measured and solutions diluted with PBS. Fibrin polymerization in chambers prefilled with DP occurred between 2 hr and 24 hr, presumably triggered by calcium supplied by the exudate from the nerve stumps. The matrix within the DP chambers was longitudinally oriented and had an apparent density proportional to the Fg concentration in the DP solution used to prefill the chamber. At all later time periods examined (i.e., 14, 16 and 18 days postimplantation - PI) a stimulation in the progress of regeneration was observed in chambers prefilled with DP containing 2.5 mg/ml Fg. For example, at 16 days PI, Schwann cell migration and the extent of axonal regeneration were 1-3 mm more advanced in the DP chambers. At 18 days PI, the process of remyelination was 3 mm more advanced and 2.5-fold more axons were present at S5 in the DP chambers. The temporal stimulation observed in chambers prefilled with DP containing 2.5 mg/ml Fg was not observed if the Fg was diluted below 2.0 mg/ml. Nor was the effect observed when chambers were prefilled with 90%, non-citrated, "intrinsically clottable" plasma which generated a randomly oriented fibrin matrix.

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367.11 AN ANTIGENIC DETERMINANT INDUCED IN THE DISTAL STUMP OF SEVERED RAT PERIPHERAL NERVE INFLUENCES AXONAL REGENERATION. W. D. Matthew and A. W. Sandrock* (SPON: R. Nishi) Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115

The distal stump of severed mammalian peripheral nerves reacts to the lesion by undergoing a series of events known as Wallerian degeneration. In the aftermath of injury, axonal and myelin debris are removed by phagocytosis and Schwann cells proliferate. Regenerating axons grow toward their target regions within the confines of the persistent Schwann cell basal laminal tubes of the distal stump. The effectiveness of axonal regeneration within this environment implies that it is particularly hospitable for regenerating axon sprouts. Richardson and Ebendal (Brain Res. 246: 57, 1982) have provided evidence that the secretion of soluble nerve growth factors is elevated in the distal stump of previously severed rat peripheral nerve. We wondered whether modifications are also made in the extracellular matrix (ECM) of peripheral nerves undergoing Wallerian degeneration so as to provide the ideal terrain for regrowing axons.

As stated in another abstract of this issue (Sandrock and Matthew), we are now able to analyze the neurite-promoting activities of rat peripheral nerve extracellular matrix components by measuring the rate of axonal regeneration from superior cervical ganglia that have been explanted onto longitudinal sections of fresh-frozen sciatic nerve. Although there is rapid axonal regrowth over sections of normal sciatic nerve, we have found that the rate of elongation is significantly greater over sections of the distal stump of peripheral nerves that had been cut four to seven days before removal (henceforth termed "reactive nerve"). In an effort to investigate the molecular basis of this phenomenon, we immunized mice with a crude membrane preparation of normal sciatic nerve, suppressed the immune response to this tissue with the anti-mitotic agent, cyclophosphamide, re-immunized with a crude membrane preparation of reactive sciatic nerve, and fused the spleen cells with a myeloma cell line. Some of the hybridomas generated by this fusion secrete monoclonal antibodies that recognize ECM determinants which are highly induced in reactive nerve, as visualized by immunofluorescence. Many of these determinants appear to be localized to the Schwann cell basal lamina. Curiously, a large number of antibodies recognizing epitopes in the ECM of sciatic nerve also stain the cell surfaces of certain CNS glial cells. One of the antibodies that binds to an inducible determinant in peripheral nerve inhibits the rate of axonal regeneration over sections of reactive nerve. We are presently characterizing the biochemical identity of this antigen, quantitating its level of induction in reactive nerve by RIA, and surveying its tissue distribution.

367.12 DYNAMICS OF INDUCTION OF AXONAL REGENERATION IN RAT OPTIC NERVE. M.J. Politis, Department of Anatomy, College of Medicine, University of Saskatchewan, Saskatoon, Canada S7N 0W0

Little or no axonal regeneration normally occurs distal to site of injury in the adult mammalian CNS. It is widely believed that this is due to creation of a millieu which is not conducive for axonal regeneration. In the present study, attempts were made to transform the matrix of traumatized rat optic nerve into a millieu which supports axonal regeneration. Specific interventions were designed to, inhibit reactive gliosis and/or, introduce exogenous tropic factor. Nerves were crushed directly behind the eyeball and a longitudinal dural slit made distally. An Elvax pellet containing homogenate obtained from distal stump of previously-transected sciatic nerve (shown in previous studies to support PNS axonal regeneration in Silastic tubes), or homogenate-free Elvax pellets were inserted into the dural slit. Reactive gliosis was regulated by systemic administration of mitotic inhibitor (Ara C, cytosine arabinofuranoside, shown in previous studies to inhibit biochemical and morphological expressions of reactive gliosis) via interperitoneal injection for the first 8 post-operative days. Axonal regeneration was assessed 2.5 mm distal to the site of optic nerve crush by, a) electron-microscopic examination, b) silver staining, and c) immunohistochemical studies using antibody neurofilament protein. Additional experiments were conducted to determine the presence of laminin in these regions.

Results in homogenate-free pellet preparations showed no evidence of axonal regeneration. In contrast, marked alterations in optic nerve were observed when homogenatecontaining pellets were employed. Approximately 50% of the otherwise reactive-glial matrix was replaced by tissue which contained large numbers of regenerating axons. EM examination showed these axons to be organized in bundles surrounded by basal laminae. These regions stained positively with silver and with antibody to neurofilament protein. This effect was dependent on the concentration of PNS homogenate in Elvax pellets, and required systemic administration of AraC. Laminin antibody staining was evident in regions associated with regenerating axons. Results suggest that CNS tissue distal to site of injury can be transformed into a millieu which will avidly support axonal regeneration by systemic administration of mitotic inhibitor and localized infusion of exogenous tropic factors/substrate molecules. Experiments are in progress to determine if exogenous laminin is sufficient or necessary for this effect and to determine if this paradym is effective in traumatized spinal cord. Supported by the MRC Grant MA-8893.

367.13 ANTISERUM DIRECTED AGAINST WHITE MATTER INDUCES THE FORMATION OF CELLULAR BRIDGES AND THE GROWTH OF AXONS WITHIN LESIONS OF THE ADULT RAT CENTRAL NERVOUS SYSTEM. E.E. Geisert, T. Darongsuwan* and C.D. Alley*. Department of Cell Biology and Anatomy, University of Alabama at Birmingham, Birmingham, AL 35294.

In previous studies (Alley and Geisert, Neurosci. Abs. 10; Geisert and Alley, Neurosci. Abs. 10), we have demonstrated that when lesions of the adult mammalian central nervous system are bathed in pepsin-digested antiserum directed against damaged brain, cellular bridges are formed that span the lesion and axons grow across the lesion in these bridges. In an initial effort to define the antibodies responsible for the bridge formation and axonal growth, different antisera were made: antiserum against white matter (made by inoculating rabbits with dissected optic nerve and fornix); antiserum directed against gray matter (made by inoculating rabbits with whole brain then absorbing with white matter); and, antiserum against scar (made by inoculating rabbits with damaged brain and absorbing with normal brain). Thus, the original antiserum was divided into three subpopulations. Rats (270 to 350g) were anesthetized, large lesions were made, a cannula was lowered into the lesion and cemented to the skull. The treatment was delivered by a mini-osmotic pump (0.5µl/hour for 14 days). The rats were assigned to treatment groups; 4 rats, normal saline; 6 rats, normal rabbit serum F(ab')2 fragments; 5 rats, anti-gray matter F(ab')2 fragments; 6 rats, anti-scar F(ab')2 fragments; and 5 rats, anti-white matter F(ab')2 fragments. Following a 20 day survival period the animals were deeply anesthetized and perfused through the heart. Three series of 40µm sections were stained by a different method: the Nissl method, a silver technique for axons, and an indirect immunoperoxidase method with the primary antibody directed against neurofilaments. The lesion area in each of the brains was reconstructed and the percentage of the lesion that was cavity, scar, or dense cellular bridge was calculated. The formation of dense cellular bridges and axonal growth was only seen in rats that were treated with the anti-white matter F(ab')2 fragments. The percentage of the lesioned occupied by dense cellular bridge in each of the anti-white matter animals was: 0%, 3.0%, 3.2%, 4.7%, and 9.9%. The response to treatment in anti-white matter animals was robust when compared to the rats from earlier studies treated with antiserum against damaged brain (in the best case only 1.6% of the lesion was bridge). The epitopes that are necessary to develop an antiserum capable of inducing bridge formation and axonal growth are found in white matter. These data demonstrate that the formation of dense cellular bridges and the axonal growth seen in this study is antibody specific, for it did not occur in rats treated with normal rabbit serum, anti-gray matter antiserum, or the anti-scar antiserum. This work was supported by the Spinal Cord Society grant number G-A UAI.

367.14 THE EFFECT OF $GM_1$, THYROXINE AND HYDROCORTISONE ON FETAL CATECHOLAMINERGIC NUCLEI TRANSPLANTED INTO THE TRANSECTED SPINAL CORD OF THE ADULT RAT. John W. Commissiong and G. Toffano. Dept. of Physiol., McGill Univ., Montreal, Canada H3G 1Y6 and Dept. of Biochem., Fidia Res. Lab., 35031 Abano Terme, Italy.

Fetal locus coeruleus (LC) of rat has previously been successfully transplanted into the transected spinal cord of the young adult rat (Commissiong, 1983). In the present experiments, the ganglioside $GM_1$, thyroxine (T4) and hydrocortisone (HC) were tested for their ability to promote the survival, growth and development of fetal day (FD) 16 LC and substantia nigra (SN) tissue after their transplantation into the transected spinal cord of the adult rat. Female Sprague Dawley rats of 150 g were anaesthetized with Brietal (60 mg/kg i.p.). A laminectomy was done, following which the cord was completely transected subpially at T8, creating a spinal cavity about one-half of a segment long. Another cavity was made at L2, in the dorsolateral intumescence. Fetal LC or SN was implanted into both cavities, and the pia over the implants sutured. The implanted animals and their appropriate controls were treated with $GM_1$ (30 mg/kg i.p.), or T4 (50-100 µmol/kg s.c.) or HC (40 mg/kg i.p.) daily for the first 21 days after surgery, and thereafter every other day until sacrificed.

As reported previously, the survival rate of the animals is 95%. The rate of fusion of the fetal implants with the spinal cord is 40%. The solid implants subdivided into smaller cell clusters which became lodged in the host tissue away from the site of implantation. Neurite and axonal growth occurred in excess of 15 mm away from the cell bodies. Growth of sympathetic noradrenergic fibres normally associated with blood vessels within the cord was again observed in the caudal region of the transected cord. Neither $GM_1$, T4 nor HC exerted any beneficial effect on any of the above parameters. However, $GM_1$ and T4 exerted a marked enhancement of the fluorescent intensity of the noradrenergic cells in the subcoeruleus which normally project to the spinal cord. The somatic dendrites of these cells were also longer, and morphologically more robust. $GM_1$ also caused an increased sprouting of the descending monoaminergic (catecholaminergic and serotonergic) damaged axons in the rostral region of the transected cord. These axotomized neurons originate in the brainstem. HC had no marked effect on any of these parameters. The results demonstrate that $GM_1$, and to a lesser degree T4 are capable of enhancing the regeneration of the chemically defined subset of neurons in the central nervous system of the adult rat. The observed effects of $GM_1$ on both the cell body and axon terminal regions of the axotomized neuron suggest that it may have a more general effect on the regenerating neuron, versus T4 for example, whose effect was restricted to the cell body region only.

Commissiong, J.W. Brain Res. 271, 174, 1983.

## SEROTONIN

368.1 Serotonin (5-HT) in the squid nervous system. Susan C. Feldman and Jean Muldur*, Dept. of Anatomy, New Jersey Medical School, Newark, N.J. 07103 and Marine Biological Laboratory, Woods Hole, MA. 02543.

As part of an ongoing inquiry into the functional, anatomical and comparative relationships of transmitter systems we describe the immunohistochemical localization of 5-HT containing neurons and processes in the nervous system of the cephalopod mollusk, the squid Loligo pealei: The nervous system of cephalopods is highly complex with separate lobes important in vision, learning and motor behavior. In addition, squid neurons range in shape from true multipolar to amacrine and the uni- and bi-polar neurons common in most invertebrates.

For this study, squid nervous system - optic lobes, circumesophageal ganglia and stellate ganglion - were dissected and fixed by immersion in Bouin's solution. Prior to sacrifice, several animals were injected with the MAO inhibitor pargyline (10mg/squid, 20 min) into the coelomic cavity. This increased 5-HT immunoreactivity primarily in fibers but did not alter the distribution of 5-HT neurons or fibers from that seen in untreated animals. 5-HT was localized in both paraffin-embedded and vibratome sections using an antiserum to 5-HT (Immunomuclear Corp.) and the standard PAP technique.

The distribution of 5-HT was extremely widespread. 5-HT neurons were confined to a small number of areas-the medulla of the optic lobe, several supraesophageal lobes, and two lobes of the subesophageal complex. In the optic lobe medulla axons of 5-HT neurons contributed to the formation of the optic tract. These could be followed into the supraesophageal ganglia where they branched extensively. In the supraesophageal complex, 5-HT neurons were found in the peduncle lobe, the frontal lobe and the anterior division of the basal lobe. In this latter lobe every cell contained 5-HT; this was in marked contrast to the other lobes in which 5-HT neurons were not clustered. In the subesophageal complex, or motor centers, 5-HT was present in a small number of cells and in fibers surrounding nonimmunoreactive neurons and in large nerves emanating from this region. In the stellate ganglion, 5-HT was limited to a small number of cells.

In summary, the squid nervous system was characterized by a widespread distribution of 5-HT fibers and a more restricted distribution of 5-HT containing neurons, an arrangement somewhat reminiscent of the mammalian situation.

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368.2 STRESS AND ACTIVITY RELATED CHANGES OF HYDROXYLASE COFACTOR CONCENTRATIONS IN BRAIN TISSUES AND BLOOD. E.H.Y. Lee. Inst. of Biomed. Sci. Academia Sinica, Taipei, Taiwan, Republic of China.

Tetrahydrobiopterin ($BH_4$) functions as a cofactor for phenylalanine hydroxylase (Kaufman 1959,1963). It also serves as a cosubstrate for brain tyrosine hydroxylase (Nagatsu et al.,1964) and tryptophan hydroxylase (Lovenberg et al., 1967) in regulation of catecholamine and serotonin biosynthesis. Previous reports have shown that amphetamine, which elevated locomotor activity, decreased striatal $BH_4$ level in rats (Lee & Mandell, 1985). Furthermore, diurnal rhythm study of rat striatal biopterin, the oxidized form of $BH_4$, indicated a high biopterin level in the light phase when activity is low and low biopterin level in the dark phase while the activity is high in these nocturnal animals (Lee & Mandell, 1985). The purposes of the present study were to examine whether there is an inverse relationship between activity level and biopterin concentration in brain and to examine the effects of electric shock stress on biopterin in different brain tissues and blood.

Thirty-three male Sprague-Dawley rats (150-225 g) were used. Twenty-one of them were used for activity and biopterin correlation study. Biopterin concentrations in the caudate nucleus and nucleus accumbens were measured in each animal. The other twelve rats were used for stress study. After 15 min of foot shock stress (2mA, 300 msec duration, 5 sec ISI on the average), animals were sacrificed immediately. Caudate, hypothalamus, hippocampus, raphe nuclei and blood were collected for biopterin assay. The method used for biopterin assay was HPLC with fluorescence detection.

Results indicate that there is a significant inverse relationship between activity and cofactor concentration in the striatum ($r=0.446, p<0.05$) but not in the nucleus accumbens ($r=0.054, p>0.05$). Foot shock stress significantly elevated cofactor levels in the hypothalamus and plasma, and decreased the same measure in the raphe nuclei without significantly changing it in the caudate nucleus and hippocampus. The above results are consistent with the finding that amphetamine reduced striatal $BH_4$ while it increased locomotor activity in animals. It also suggests that endogenous striatal $BH_4$ is an important factor regulating animal's locomotor activity in general. Alterations of $BH_4$ levels in specific brain regions and blood may also be a new indicator of stress situations. Change of $BH_4$ in the hypothalamus may be associated with the neurotransmitter-hormonal interrelationships along the adenohypophyseal axis which regulates the release of stress hormones. This work was supported by the research fund of the Inst. of Biomed. Sci., Academia Sinica, Taiwan, R.O.C.

368.3 SPIROXATRINE: A SELECTIVE ANTAGONIST OF SEROTONIN-1A RECEPTORS. D.L. Nelson and E.W. Taylor*. Dept. of Pharmacol. & Toxicol, Col. of Pharmacy, Univ. of Arizona, Tucson, AZ 85721.

The characterization of the properties and functional significance of the putative subtypes of serotonin (5-hydroxytryptamine, 5HT) receptors has often been impeded by the lack of selective pharmacologic agents, especially selective antagonists for the 5HT-1 receptors. Since the neuroleptic spiperone has relatively high-affinity for the 5HT-1A subtype of sites (in addition to its high affinity for 5HT-2 sites), we have examined several analogs of this compound in an attempt to find a structure that would retain the high affinity for the 5HT-1A site, but which would have lowered affinity for the 5HT-2 site. The relative potencies of the compounds at the different 5HT binding sites were assessed by comparing the inhibition curves generated against the binding of [$^3$H]5HT (which labels both 5HT-1A and 5HT-1B sites), [$^3$H]8-hydroxy-N,N-dipropyl-2-aminotetralin (PAT, which labels 5HT-1A sites), and [$^3$H]ketanserin (KET, which labels 5HT-2 sites). The binding was carried out using membranes prepared from the rat cerebral cortex. The concentrations of the [$^3$H]ligands were approximately equal to their reported $K_d$ values (1.8 nM, 1.0 nM, and 0.5 nM for [$^3$H]5HT, [$^3$H]PAT, and [$^3$H]ketanserin respectively). Of the compounds tested only one, spiroxatrine (Janssen Pharmaceutica, Beerse , Belgium), showed selectivity. The $IC_{50}$ values (nM) for this compound compared to spiperone were as follows:

| | [$^3$H]PAT | [$^3$H]5HT | [$^3$H]KET |
|---|---|---|---|
| Spiperone: | 137.6 | 4038 | 0.68 |
| Spiroxatrine: | 5.04 | 1211 | 226.2 |

These data showed that spiroxatrine, unlike spiperone, was much more potent at 5HT-1A than 5HT-2 sites. In addition, its low $IC_{50}$ at [$^3$H]5HT compared to [$^3$H]PAT binding suggested a very low affinity for the 5HT-1B site. When spiroxatrine was examined in an in vitro preparation of the canine basilar artery, which has been proposed to contain 5HT-1 receptors, it produced no contractions up to the highest concentration used ($10^{-5}$ M). However, when 5HT was used as the agonist (in the presence of 100 nM KET to eliminate the possibility of 5HT-2 receptors contributing to the contraction), spiroxatrine antagonized the contractions with a $K_B$ value of 25 ± 8 nM. Arterial contractions produced by low concentrations (<$10^{-6}$ M) of PAT were also blocked by spiroxatrine (100 nM). The data, therefore, suggest that spiroxatrine is a selective 5HT-1A antagonist. Because of its high affinity this compound may have significant potential as a tool for studying these sites. (Supported by NIH grant NS16605 and a predoctoral fellowship from the PMA Foundation).

368.4 MCPP, A 5HT IB AGONIST, PRODUCES PROLACTIN, CORTISOL AND GROWTH HORMONE RELEASE AND BIPHASIC BEHAVIORAL CHANGES IN HEALTHY HUMAN SUBJECTS. G. R. HENINGER and D. S. CHARNEY*, Dept. of Psychiatry, Yale Univ. Med. Sch., New Haven, CT 06508.

Recent biochemical and physiologic investigations have provided evidence of two different serotonin (5HT) type I receptors. 5HT Ia receptors preferentially respond to indole agonists, and 5HT Ib receptors to piperazine agonists. In order to assess the effects of 5HT Ib stimulation in humans, the specific 5HT Ib agonist MCPP (1-(3-chlorophenyl) piperazine) and the 5HT precursor tryptophan (TRYP) were administered intravenously to healthy subjects. This allowed a comparison of the effects of specific 5HT Ib stimulation by MCPP to the relatively less specific 5HT stimulation resulting from the tryptophan infusion.

METHODS: Thirteen subjects (5 males) between the ages of 18 and 47 gave informed written voluntary consent and received placebo, .025, .05, .1, and .2 mg/kg of MCPP and 100 mg/kg L-tryptophan on 6 separate test days. Subjects slept at home, fasted overnight and reported to the laboratory at 8:30 A.M. where they had a catheter inserted, then a 90 min. adaptation period, and then were administered one of the infusions. Repeated blood sampling and subjective and objective assessment of behavior were carried out before and after the infusion. Plasma prolactin (PRL), cortisol (CORT) & growth hormone (GH) were assayed with standard RIA methods.

RESULTS: MCPP produced a significant dose response curve for all three hormones. At the .1 mg/kg dose the average maximal effect (peak minus base) was 12 ng/ml, 10 ug/dl, and 8 ng/ml for PRL, CORT and, GH, respectively. The TRYP infusion produced a corresponding maximal effect of 18 ng/ml, ~2 ug/dl and 7 ng/ml for PRL, CORT and GH respectively. MCPP produced increased feelings of mellow, high, happy and calm at the .05 mg/kg dose and several subjects had increased sexual desire. At .1 and .2 mg/kg of MCPP, many subjects had increased dysphoric feelings of anxious, nervous, irritable and drowsy and somatic symptoms of nausea, dizziness, blurred vision, tremulousness, piloerection, hot and cold flashes, feelings of unreality and headache. This prevented use of the .2 mg/kg dose in 6 subjects. TRYP produced mild increases in feelingcs of drowsy, high and mellow without the somatic symptoms seen with MCPP. Blood pressure, pulse rate and oral temperature demonstrated little change with either MCPP or TRYP.

DISCUSSION: MCPP and TRYP both stimulated PRL and GH release; while only MCPP stimulated CORT release. MCPP had a biphasic effect on mood, producing positive feelings at low doses and negative feelings at higher doses. TRYP did not produce negative feelings. This data suggests that the neuroendocrine and behavioral response to MCPP will be a useful test of the 5HT Ib receptor system for the study of neurologic and psychiatric conditions. Supported by USPHS MH36229, MH25642, and MH30929.

368.5 SOLUBILIZATION OF SEROTONIN 5-HT1c SITES FROM PIG CHOROID PLEXUS. P.R. Hartig, and K.A. Yagaloff*, Department of Biology, The Johns Hopkins University, Baltimore, MD 21218.

We have previously reported the localization of a unique serotonergic binding site in the rat choroid plexus and have characterized the binding of $^{125}$I-LSD to rat choroid plexus homogenates (Yagaloff and Hartig, J. Neuroscience (1985)). In addition, Pazos et al. (Eur. J. Pharmacol. 106, 539 (1984)) have characterized a very similar serotonergic binding site (termed 5-HT1c) in pig choroid plexus homogenates. We report here the solubilization of 5-HT1c serotonergic binding sites from pig choroid plexus membranes using ionic and nonionic detergents.

Pig choroid plexus membrane preparations were solubilized with various detergents and centrifuged at 100,000 x g for 1 hour. Approximately 40% of the membrane sites were recovered in the soluble supernatant using a postlabelling methodology (reversible labelling after solubilization). Specific binding was typically 70-80% of total labelling in the solubilized supernatant. Binding to the solubilized site was reversible, stereospecific, and both heat and protease sensitive. The solubilized sites were not retained by a 0.22 micron Millipore filter and were exluded from the void volume of a Sepharose CL-6B column.

Scatchard analyses of binding to the membrane preparation and to the solubilized supernatant indicated that radioligand binding affinities were the same in both preparations. The pharmacological profile of binding to the choroid plexus site revealed an identical rank order of potency for 12 compounds binding to both solubilized and membrane sites. In addition, nearly all of the compounds were equipotent or more potent in binding to the solubilized site in comparison to the membrane site.

The yield and stability of the solubilized 5-HT1c site are superior to those obtained for other solubilized serotonergic sites in the brain, indicating that the choroid plexus 5-HT1c site is a promising system for the purification and biochemical characterization of mammalian brain serotonergic sites.

368.6 COVALENT LABELING OF PUTATIVE SEROTONIN RECEPTOR IN APLYSIA NEURONS. J.C. Shih, T. Saitoh, R.W. Ransom* and J.H. Schwartz (Spon. S. Eiduson ), Institute for Toxicology, School of Pharmacy University of Southern California, Los Angeles, CA 90033, and Howard Hughes Medical Institute, Columbia University, College of Physicians and Surgeons, New York, NY 10032.

We have previously shown that 1-(2-4-aminophenyl)ethyl)-4-(3-trifluoromethyl phenyl) piperazine (p-$NH_2$-PE-TFMPP) selectively binds to 5-HT-1A receptor in rat brain (Asarch, Ransom and Shih, Life Sci. 36:1265-73, 1985). The accompanying abstracts show that $^3$H-p$NH_2$-PE-TFMPP binds selectively with high affinity to 5-$HT_{1A}$ receptor sites in rat brain. Furthermore, the azido derivative of $^3$H-p-$NH_2$-PE-TFMPP ($^3$H-p-azido-PE-TFMPP) photolabels one 5-HT-1A receptor protein in rat brain. This report demonstrates the covalent labeling of serotonin receptor in Aplysia neurons by $^3$H-p-azido-PE-TFMPP.

Serotonin increases the synthesis of c-AMP in Aplysia neurons and stimulates the adenylate cyclase in membranes isolated from nervous tissue with a Kact of 0.8 uM. Under the same conditions, p-$NH_2$-PE-TFMPP stimulates adenylate cyclase with a Kact of 20 uM. p-azido-PE-TFMPP also stimulates the cyclase in the dark, however, with much less efficiency (Kact = 200 uM). Irradiation of the membrane with 50 uM of p-azido-PET abolished 75% of the cyclase activity stimulated by 50 uM serotonin suggesting that this compound may occupy the receptor binding site, and thus may be suitable for labeling the receptor protein. $^3$H-azido-PE-TFMPP (50 uM) was incubated with membranes from Aplysia neurons for 5 min at 25° C, washed once, and irradiated on ice. A fluorogram of SDS-gels shows that there are many proteins labeled under this condition, however, only one polypeptide with a molecular weight of 63,000, whose labeling was protected by the presence of 0.2 uM serotonin during incubation and photolysis. None of the protein labeled by $^3$H-azido-PE-TFMPP in Aplysia muscle could be protected by serotonin. This result suggests that the protein with M.W. of 63,000 may be related to serotonin receptors. (Supported in part by grant from NIMH, MH 37020.)

368.7 IDENTIFICATION OF A 5-$HT_{1A}$ RECEPTOR POLYPEPTIDE IN RAT BRAIN BY PHOTOAFFINITY LABELING. R.W. Ransom*, K.B. Asarch*, J.C. Shih (Spon. S. Chen), Institute for Toxicology, School of Pharmacy, University of Southern California, Los Angeles, CA 90033.

The reversible and irreversible binding of 1-(2-(4-azidophenyl) ethyl)-4-(3-trifluoromethylphenyl) piperazine (pAzido-PE-TFMPP) and ($^3$H)pAzido-PE-TFMPP to rat brain 5-$HT_{1A}$ receptors is presented. The inhibition of ($^3$H)5-HT binding in cortex and hippocampus by pAzido-PE-TFMPP was best fit by a two site model. The computer generated dissociation constants for the azide's high and low affinity sites were 0.4 nM and 200 nM, respectively. The fraction of high affinity sites (40% in cortex, 60% in hippocampus) for the azide corresponded to the number of 5-$HT_{1A}$ sites in these tissues as determined by spiperone displacement of ($^3$H) 5-HT binding. pAzido-PE-TFMPP inhibition of ($^3$H)5-HT binding in the presence of 1 uM spiperone was consistent with displacement from a single site having a Kd of 200 nM for the azide. These results demonstrate that pAzido-PE-TFMPP and spiperone recognize the same two subpopulations of 5-$HT_1$ sites and it is the 5-$HT_{1A}$ subtype for which pAzido-PE-TFMPP has high affinity.

Using 10 uM 5-HT to define nonspecific binding, ($^3$H)pAzido-PE-TFMPP labeled a single site in membranes prepared from cortex and hippocampus with a Kd of 1 nM. The ability of 5-HT, LSD, 8-hydroxy-2-(di-n-proplyamino)tetralin (PAT), and spiperone to displace specific ($^3$H)pAzido-PE-TFMPP binding corresponded to their high affinity for 5-$HT_{1A}$ sites. In photolysis experiments, hippocampal membranes were incubated with 6 nM ($^3$H)pAzido-PE-TFMPP, washed twice, and irradiated. SDS-PAGE of these membranes showed that radioactivity was primarily incorporated into a polypeptide with a molecular weight of 54,000. The labeling of this polypeptide was completely inhibited when membranes were incubated with the tritiated azide in the presence of 1 uM 5-HT, PAT, LSD, or spiperone. The labeling was unaffected by the presence of 1 uM ketanserin. These results indicate that the labeled polypeptide represents the 5-$HT_{1A}$ receptor or a subunit of the receptor. (Supported by NIMH Grant No. MH 37020.)

368.8 [$^3$H]-1-(2-(4-aminophenyl)ethyl)-4-(3-trifluoromethylphenyl) Piperazine: A Selective Radioligand for 5-$HT_{1a}$ Receptors in Rat Brain. K.B. Asarch*, R.W. Ransom*, and J.C. Shih. Institute for Toxicology, School of Pharmacy, University of Southern California, Los Angeles, California 90033.

We have synthesized a new serotonergic radioligand which has high selectivity for 5-$HT_{1a}$ sites in rat brain. 1-(2-(4-amino-phenyl)ethyl)-4-(3-trifluoromethylphenyl)piperazine ($pNH_2$-PE-TFMPP) inhibits [$^3$H]5-hydroxytryptamine binding to 5-$HT_{1a}$ and 5-$HT_{1b}$ sites in rat brain with apparent equilibrium dissociation constants ($K_d$) of 2.9 nM and 328 nM, respectively. $pNH_2$-PE-TFMPP was tritiated by catalytic reduction of a ring brominated analogue of $pNH_2$-PE-TFMPP under tritiated hydrogen gas. Using either 10 μM serotonin or 1 μM 8-hydroxy-2-(di-n-propylamino)tetralin (DPAT) to define nonspecific binding, [$^3$H]$pNH_2$-PE-TFMPP bound to a single class of sites in rat cortical membranes with a $K_d$ of 1.8 nM and a maximal binding density ($B_{max}$) of 222 fmol/mg protein. This affinity very closely matched the affinity obtained by displacement with the unlabeled $pNH_2$-PE-TFMPP (2.9 nM). d-Lysergic acid diethylamide and serotonin, two nonselective inhibitors of [$^3$H]5-HT binding, displaced 1 nM [$^3$H]$pNH_2$-PE-TFMPP with $K_i$ values (2.5 nM and 18 nM, respectively) that are consistent with their affinities for 5-$HT_1$ receptors. Spiperone and DPAT, two compounds that discriminate [$^3$H]5-HT binding to 5-$HT_{1a}$ and 5-$HT_{1b}$ sites, inhibited [$^3$H]$pNH_2$-PE-TFMPP binding with $K_i$ values (40 nM and 2.8 nM, respectively) in accordance with their much higher affinities for the 5-$HT_{1a}$ receptor subtype. Furthermore, the ability of (m-trifluoromethylphenyl) piperazine and ketanserin to inhibit [$^3$H]$pNH_2$-PE-TFMPP binding ($K_i$ values 233 nM and 1000 nM, respectively) reflected their low affinities for the 5-$HT_{1a}$ receptor. The regional distribution of serotonin sensitive [$^3$H]$pNH_2$-PE-TFMPP sites also correlated with the relative densities of 5-$HT_{1a}$ receptors in the rat brain: hippocampus > cortex > corpus striatum > cerebellum). These results indicate that [$^3$H]$pNH_2$-PE-TFMPP binds selectively and with high affinity to 5-$HT_{1a}$ receptor sites in the rat brain.

In the rat hippocampus the divalent cations magnesium, calcium, and manganese at 5 mM all significantly increase the amount of specific [$^3$H]$pNH_2$-PE-TFMPP binding (66 to 99%) as has been observed for [$^3$H]5-HT binding. In contrast, GTP decreases the specific binding of both [$^3$H]$pNH_2$-PE-TFMPP and [$^3$H]5-HT specific binding in the rat hippocampus with similar potencies ($IC_{50}$ = 30 μM). Preliminarily, we observe a stimulation of adenylate cyclase activity by $pNH_2$-PE-TFMPP in synaptic membranes prepared from hippocampi from adult rats of about two-thirds the magnitude of the 5-HT stimulation with an $EC_{50}$ (100 nM) similar to 5-HT. (Supported by NIMH Grant No. RO1-MH 37020).

368.9 DIRECT LABELING OF 5-$HT_{1A}$ AND 5-$HT_{1B}$ BINDING SITES WITH $^3$H-OH-DPAT AND $^3$H-5HT IN BOVINE BRAIN. S. J. Peroutka, P. J. Ison* and J. R. Schlegel*. Department of Neurology, Stanford University Medical Center, Stanford, CA 94305.

The interactions of 12 serotonergic agents with both $^3$H-8-OH-DPAT binding in bovine hippocampal membranes and $^3$H-5-HT binding in striatal membranes were analyzed. 8-OH-DPAT is the most potent inhibitor of $^3$H-8-OH-DPAT binding. 5-HT, bufotenine, 5-MeDMT, RU 24969 and TVX Q 7821 also have nanomolar affinity for the $^3$H-8-OH-DPAT binding site. DMT, TFMPP, spiperone and pizotifen have $IC_{50}$ values ranging from 190 - 750 nM. Quipazine is the weakest agent at the $^3$H-8-OH-DPAT binding site with an $IC_{50}$ value of 4,500 ± 1,000 nM. These values correlate well with drug affinities for $^3$H-8-OH-DPAT binding sites in rat brain membranes.

A strikingly different pharmacological profile is observed at the $^3$H-5-HT binding site in bovine striatal membranes. 5-HT ($IC_{50}$ = 7.5 ± 2 nM) and bufotenine ($IC_{50}$ = 13 ± 3 nM) are the only agents which display less than 100 nanomolar affinity for these binding sites. These drugs, in addition to DMT, TFMPP and quipazine, have similar affinity for both hippocampal $^3$H-8-OH-DPAT and striatal $^3$H-5-HT binding sites. All other agents are markedly less potent at $^3$H-5-HT striatal binding sites than $^3$H-8-OH-DPAT hippocampal binding sites. Drugs such as pizotifen, spiperone and RU 24969 are 19 - 86-fold less potent at $^3$H-5-HT striatal binding sites. For example, spiperone is 86-fold less potent at $^3$H-5-HT ($IC_{50}$ = 18,000 ± 2,000 nM) binding sites than $^3$H-8-OH-DPAT sites. TVX Q 7821 and buspirone are more than 1,000-fold less potent at these binding sites. 8-OH-DPAT is the most selective agent, being 18,000-fold weaker ($IC_{50}$ = 31,000 ± 6,000 nM) at $^3$H-5-HT binding sites in the striatum than at $^3$H-8-OH-DPAT binding sites in the hippocampus.

The major finding of the present study is that 5-$HT_{1A}$ and 5-$HT_{1B}$ binding sites can be selectively labeled using specific ligands in appropriate brain regions. $^3$H-8-OH-DPAT can be used to directly label the 5-$HT_{1A}$ binding site in hippocampal membranes. Although no specific label exists for the 5-$HT_{1B}$ binding site, Nelson and co-workers have defined the site as $^3$H-5-HT sites which are insensitive to low concentrations of spiperone. In addition, lack of sensitivity to 8-OH-DPAT is also a characteristic of the 5-$HT_{1B}$ binding site (Middlemiss and Fozard, 1983). According to these criteria, 85 - 90% of the $^3$H-5-HT labeled sites in bovine striatal membranes can be classified as 5-$HT_{1B}$ binding sites. Therefore, analysis of $^3$H-5-HT binding in this tissue represents the most direct method to characterize the 5-$HT_{1B}$ site.

368.10 NUCLEOTIDE INTERACTIONS WITH 5-$HT_{1A}$ AND 5-$HT_{1B}$ BINDING SITES. J. R. Schlegel* and S. J. Peroutka. (SPON: R. Angel). Department of Neurology, Stanford University Medical Center, Stanford, California 94305.

Nucleotide interactions have been extensively evaluated at 5-$HT_1$ binding sites. However, $^3$H-5-HT binding sites have been recently subdivided into 5-$HT_{1A}$ and 5-$HT_{1B}$ binding site subtypes. Using $^3$H-8-OH-DPAT to directly label 5-$HT_{1A}$ binding sites and $^3$H-5-HT in the presence of $10^{-7}$M 8-OH-DPAT to label 5-$HT_{1B}$ binding sites in rat cortex, we have evaluated the interactions of guanine and adenine nucleotides with 5-$HT_1$ binding site subtypes.

Guanosine triphosphate (GTP) and guanosine diphosphate (GDP) decrease the specific binding of $^3$H-8-OH-DPAT to 5-$HT_{1A}$ binding sites. At a concentration of $10^{-5}$M, GTP and GDP reduce specific $^3$H-8-OH-DPAT binding to 83 ± 2% and 88 ± 5% of control values, respectively. At $10^{-4}$M, GTP and GDP reduce specific $^3$H-8-OH-DPAT binding to 49 ± 4% and 50 ± 3%. At $10^{-3}$M, they reduce specific binding to 21 ± 2% and 35 ± 3%. By contrast, guanosine monophosphate (GMP) and the adenine nucleotides have little effect, reducing specific $^3$H-8-OH-DPAT binding to 85 - 92% of control values at concentrations of $10^{-4}$M.

At $^3$H-8-OH-DPAT binding sites, the affinity of both classical and novel serotonergic agonists (5-HT, 5-MT, 5MeODMT, LSD, TFMPP, 8-OH-DPAT, RU24969, buspirone, and TVX Q 7821) is decreased in the presence of $10^{-4}$M GTP, with $IC_{50}$ values shifted two to four fold. No significant shift in displacement curves or $IC_{50}$ values is observed in the presence of GTP when serotonergic antagonists (spiperone, metergoline, and pirenperone) compete for 5-$HT_{1A}$ binding sites. Saturation experiments using $^3$H-8-OH-DPAT in the absence or presence of $10^{-3}$ and $10^{-4}$M GTP demonstrates a decrease in the affinity of $^3$H-8-OH-DPAT for 5-$HT_{1A}$ binding sites in rat cortex without affecting the total number of binding sites.

By contrast, less pronounced effects of GTP and GDP are observed with $^3$H-5-HT binding to 5-$HT_{1B}$ binding sites. In rat cortex, $^3$H-5-HT binding in the presence of $10^{-7}$M 8-OH-DPAT is reduced to 80 ± 4% and 81 ± 4% by $10^{-4}$M GTP and $10^{-4}$M GDP, respectively. GMP and the adenine nucleotides have little effect at 5-$HT_{1B}$ binding sites, with specific $^3$H-5-HT binding ranging from 89 - 100% in the presence of $10^{-4}$ nucleotide.

The major finding of the present study is that guanine nucleotides differentially affect radioligand binding to 5-$HT_1$ binding site subtypes. Although GTP and GDP versus other nucleotides selectively inhibit agonist binding to both 5-$HT_{1A}$ and 5-$HT_{1B}$ sites, the effect is significantly more pronounced at the 5-$HT_{1A}$ binding site.

369.1 CAN TRIGEMINAL CHEMORECEPTORS DISCRIMINATE ODORANTS? W.L. Silver, J.R. Mason*, and A.H. Arzt*, Monell Center, Phila., PA 19104

Trigeminal chemoreceptors can respond to a wide variety of odorants. However, it is not known whether these chemoreceptors can discriminate between odorants matched for equal intensity. We addressed this issue using both electrophysiological and behavioral techniques with the tiger salamander.

For the electrophysiological experiments, integrated multiunit responses were obtained from the trigeminal nerve as it passed through the orbit of the eye. An air-dilution olfactometer (as was also used in the behavioral experiments) delivered the stimuli to the animals. Concentration-response curves were obtained for amyl acetate (AMA) and cyclohexanone (CH). The concentration of both compounds necessary to produce an equivalent response (150% of CH std., ~1100 ppm) was then used in cross-adaptation experiments. A background stream of ~1700 ppm CH or ~ 2800 ppm AMA severely reduced the responses to both AMA and CH. Only concentrations above background elicited increases in response magnitude.

For the behavioral experiments, six salamanders were randomly assigned to two groups. Group 1 was trained to avoid CH (S+: ~ 900 ppm) and not to respond to AMA (S-: ~1100 ppm). Group 2 received opposite training. When criterion was achieved (> 90% S+ responding, <20% S- responding), both groups were given varied concentrations of their respective S+ odorant. S+ concentrations (CH:~ 325 ppm; AMA: ~200 ppm) that elicited 80% avoidance were used in subsequent discrimination tests. Both groups discriminated CH from AMA.

Following discrimination trials, all animals were given bilateral olfactory nerve cuts, and additional presentations of varied S+ concentrations. Higher concentrations of odorant were necessary to elicit 80% avoidance in nerve cut animals. Discrimination tests between behaviorally equivalent odorant concentrations (CH: ~1000 ppm; AMA ~1250 ppm) revealed that neither group was able to discriminate CH from AMA. Additional discrimination trials with concentrations that elicited 95% avoidance produced similar results. However, both groups discriminated an S+ concentration that elicited 95% avoidance from an S- concentration that elicited 80% avoidance (for the opposite group). When concentrations were switched (i.e., 80% S+, and 95% S-), all animals avoided S- (and not S+) presentations.

On the basis of these electrophysiological and behavioral findings, we speculate that trigeminal chemoreceptors are unable to discriminate between odorants matched for equal intensity. Thus, these chemoreceptors discriminate odorant quantity, not quality.

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369.2 THE PHEROMONAL RELEASE OF NIPPLE-SEARCH BEHAVIOR IN NEWBORN RABBITS DOES NOT DEPEND ON THE VOMERONASAL ORGAN R. Hudson* and H. Distel* (SPON: A. Hofbauer). Inst. Med. Psychol., Univ. München, D-8000 München, Germany.

Rabbit pups are only nursed for about 3 minutes once a day. To locate nipples in this short time they are totally dependent on a short-ranging pheromone on the mother's belly which releases and guides the stereotyped nipple-search behavior. This is the case even for pups delivered preterm, and thus without postnatal experience of the pheromone. However, newborn pups can be rapidly conditioned to respond to artificial odors with nipple-search behavior when these are paired with suckling (Hudson, Dev.Psychobiol., in press). As it has long been known that bilateral olfactory bulbectomy completely disrupts suckling behavior in rabbits (v. Gudden, Arch. Psychiat. 2:693, 1870), the relative role of the main and accessory olfactory systems in mediating inborn and acquired responsiveness to nipple-search odors was investigated.

Irrigating the nasal mucosa of 3-day-old pups (n=5) with a 2% zinc sulfate solution eliminated responsiveness to the pheromone, and hence suckling, on the subsequent days. Similarly, pups conditioned to the odor of citral (n=6) no longer responded to it with nipple-search behavior after the ZnSO4 treatment. While histological verification of these cases showed the olfactory epithelium to be completely destroyd, the epithelium of the vomeronasal organ appeared virtually intact.

In contrast, completely removing the vomeronasal organ of normal (n=4) and citral-conditioned (n=3) pups on day 3 had little effect on their ability to respond to the pheromone, or to the learned odor, with nipple-search behavior. Even pups delivered 1 day preterm by injecting the doe with oxytocin (n=5), and lesioned before their first suckling experience, responded as vigorously to the pheromone as unlesioned control pups.

These findings suggest that both inborn and postnatally acquired responsiveness to odors releasing nipple-search behavior in newborn rabbits are mediated by the main olfactory system. This is of particular interest given the important role attributed to the accessory olfactory system in pheromonal perception. However, it might be necessary to distinguish between pheromones associated with suckling and therefore peculiar to mammals, and other pheromones.

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369.3 **CYTOARCHITECTURE AND PROJECTION SITES OF SOME RETROBULBAR CORTICES IN THE RAT BRAIN.** C.K.H. REYHER and W.K. SCHWERDTFEGER. Dept. Neuroanatomy, Freie Universität Berlin, D - 1000 Berlin 33, FRG Max-Planck-Institut für Hirnforschung, Frankfurt. (SPON: European Neuroscience Association).

The organization of the central connectivity of individual olfactory-related retrobulbar structures with the primary and higher olfactory brain was investigated by means of highly sensitive tracing methods. To characterize the afferent and efferent olfactory connections of the retrobulbar cortices wheat germ agglutinin conjugated to horseradish peroxidase (Sigma) was injected stereotaxically into the main and accessory olfactory bulb, into the anterior piriform and dorsal peduncular cortex, and into several subdivisions of the anterior olfactory nucleus. After a 1-day survival time the animals were transcardially perfused with a 1% paraformaldehyde/ 1% glutaraldehyde - solution. Frozen sections were reacted with tetramethylbenz - idine as chromogen.

Neurons projecting to individual retrobulbar cortices were found to arise in the subiculum, the ventral limb of the precommissural hippocampus, the lateral entorhinal cortex, the anterior and posterior parts of the piriform cortex, the tuberculum olfactorium, the entire main olfactory bulb, as well as the mesencephalic raphe nuclei and the locus coeruleus. Some of these tracts were topographically mapped by a computerized morphometric unit to define localization and arborization of individual neurons; this allowed comparison of HRP-labeled cells with corresponding ones obtained by using Golgi impregnation techniques. Thus, we determined e.g. that the majority of output neurons of the lamina externa of the anterior olfactory nucleus which project exclusively to the opposite olfactory bulb are characterized by a lack of basal dendrites.

These results offer evidence that retrobulbar cortices are involved in a highly organized controlling system which may exert influences onto the parallel processing of olfactory information related to the two hemispheres.

369.4 TASTE BUD DENSITY ON THE HUMAN TONGUE. Inglis J. Miller, Jr. Dept. of Anatomy, Bowman Gray Sch. Med., Winston-Salem, NC 27103.

The density and regional distribution of human fungiform taste buds are important factors in the diagnosis of taste disorders and the study of taste perception. While such information is available for several species of animals, equivalent observations for human tongues is lacking. Preliminary studies are underway on tongues from 37 human cadavers as follows: 5 infants, 4 aged 20-39 yrs, 7 aged 40-59 yrs,8 between 60-75 yrs,10 from 75 to 95 yrs and 3 of unknown origin. Photography was used to document morphological features of the surface. Areas of the tongue tip and midregion measuring about 1-2 cm sqr were sectioned serially, mounted on slides, stained with H & E, and examined by light microscopy. Preliminary results are available for males from 5 adult and one infant subject aged 79, 53, 29, 22, 22 yrs and 2 mo., respectively. The number of papillae containing taste buds from adults varied from 0 in the midregion of one specimen to 55 in the tip region of another. The tip contained an average of 18.2 gustatory papillae per cm sqr of surface, while the midregion contained only about 6.8 gustatory papillae per cm sqr of surface. Taste buds were observed on eminences which differed from classical fungiform papillae, so there was no effort to count the number of "papillae" which lacked taste buds. From 1 to 18 taste buds were found on each gustatory papilla for an average of 4.8 taste buds/papilla on the tip and 3.8 taste buds per papilla on the midregion. The density of taste buds per cm sqr of surface on the tip ranged from 3.6 to 170.8 with a mean of 100.5. The density in the midregion ranged from 0 to 68.7 taste buds per cm sqr with a mean of 27.0 across subjects. The infant tongue contained 157 gustatory papillae on an area of 0.8 cm sqr on the tip for a density of 196 papillae/cm sqr. These papillae contained 328 taste buds (410 taste buds/cm sqr) for an average of 2.09 taste buds/papilla with a range from 1-6. The midregion contained 68 gustatory papillae on a surface of 1.08 cm sqr for a density of 63 papillae per cm sqr. There were 152 taste buds for an average of 2.2 (s.d. 1.3, range 1-7) taste buds per papilla. Thus, the average number of taste buds per papilla was higher for the adults than the infant, but the density of taste buds was higher on the tongue of the child which contained only about 1/3 as much surface area. The two youngest adults (both 22 yrs) had lower taste bud densities than the three older ones and the infant. Both died unexpectedly of acute trauma. We plan to examine gender and race in subsequent studies as well as to document relevant findings from autopsy and medical history. Our objective is to determine taste bud density in a population of human subjects and to identify sources of variation within the population.(This work is supported by grant NS 20101 from NINCDS and by donations of anatomical gifts.)

369.5 CONVERGENCE OF SYNAPTIC INPUT IN MOUSE TASTE BUDS. J.C. Kinnamon and T. Sherman*. Rocky Mountain Taste and Smell Center, Univ. of Colorado Medical School and Dept. MCD Biology, Univ. of Colorado, Boulder, CO 80309.

We have previously demonstrated that both light cells and dark cells synapse onto sensory nerve fibers in murine taste buds. In order to determine the patterns of synaptic connectivity, we have been using the combination of high voltage electron microscopy and three-dimensional computer reconstructions from serial thick sections to study sensory nerve arborizations and synaptic connections in murine vallate taste buds. Nerve fiber contours and the locations of taste cell-sensory nerve synapses have been digitized into a microcomputer using software developed by Dr. Stephen Young (Univ. of CA, San Diego) and Mr. Timothy Edwards (CO State Univ., Fort Collins). To date we have reconstructed 5 nerve fibers and their synaptic connections.

Nerve fibers enter the taste bud at its base and course tortuously up to within 6 um of the taste pore. Some nerve fibers have no branches while others form complex branching patterns extending from one side of the taste bud to the other. A single nerve fiber may have up to 7 synapses with taste bud cells. In all instances the taste bud cells are the presynaptic elements. We have observed two instances where a taste cell has had 3 synapses onto the same nerve fiber.

Some nerve fibers receive synaptic input from light cells or light cells and intermediate cells. Other nerve fibers receive input from dark cells or dark cells and intermediate cells. In no case does a nerve fiber receive input from both dark and light cells.

We are currently in the process of reconstructing more nerve fibers. Although our sample size is small, we speculate that some form of recognition exists, wherein nerve fibers form synapses with specific taste cell types. The basis for this specificity is unknown, but the functional implications are apparent: Separate pathways for dark cells and light cells support the notion that these different cell types may respond differentially to the various types of gustatory stimuli. Hence, some form of range fractionation may exist among the gustatory receptor cells in taste buds.

This work was supported in part by a grant from the Procter & Gamble Co. and NIH grant RR-00592.

369.6 THE GUSTATORY RECIPIENT ZONE OF THE NUCLEUS OF THE SOLITARY TRACT IN THE HAMSTER. B.J. Davis and T. Jang*. Department of Cell Biology and Anatomy, University of Alabama at Birmingham, AL 35294.

A series of correlative light and electron microscopic studies have revealed the gustatory zone of the hamster's nucleus of the solitary tract (NST), and have characterized its major peripheral inputs and neuronal types. Our previous work has quantified the axonal constituencies of the chorda tympani (CT) (Jang and Davis, Chem. Sen. Abs., '85). The IXth nerve contains about 1600 fibers and averages 96μm in diameter. The cross-sectional area of the IXth nerve is at least twice that of the CT. 80% of the IXth nerve fibers are unmyelinated and average 0.6μm in diameter; the remaining myelinated fibers average 1.8μm. Our quantitative light morphometric and accompanying EM studies find four common and morphologically distinct classes of neurons in the gustatory NST based on either differences in cross-sectional somal areas (and major and minor diameters), their general appearances and ultrastructural features. X1 neurons represent 52% of our sample (N=866), possess deeply invaginated nuclei and have somal areas that average 187 μm2; X3 neurons appear similar to the X1 group, are encountered only 22% of the time and have smaller somal areas of 99 μm2. In contrast, 12% of our sample are X2 neurons which do not possess invaginated nuclei, have relatively large somal areas averaging 201 μm2, and possess an unusual amount of perinuclear cytoplasm and membraneous organelles. The smallest class of gustatory NST neurons are the X4 group; this group also has non-invaginated nuclei, has somal areas of only 82 μm2 and almost no perinuclear cytoplasm, are comparable in size to olfactory bulb granule cells, and are good candidates for interneurons. Output neurons have been identified at the EM level following HRP injections into the pontine taste area, the rostral projection target of the gustatory NST. These HRP-positive neurons are most often large and possess invaginated nuclei and are classified as X1 neurons, although invaginated HRP-positive neurons comparable in size to X3 neurons are also observed. Larger non-invaginated X2 neurons represent about 21% of the HRP-positive neurons observed. HRP-positive X4 neurons have not been seen. Our previously described light microscopic morphometric and ultrastructural studies of the gustatory NST, together with the above comparison of the morphologically distinct neurons of the gustatory NST at both the light and EM microscopic levels contribute to our understanding of the intrinsic organization of a major relay nucleus in gustation.

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369.7 PROJECTIONS TO THE GUSTATORY THALAMUS OF NEW WORLD PRIMATES. R.B. Hamilton, T.C. Pritchard, and R. Norgren. Dept. Behavioral Science, Col. of Medicine, Penn. St. Univ., Hershey, PA 17033.

The central organization of the gustatory system differs between rodents and lagomorphs, on the one hand, and non-human primates, on the other. In rodents, gustatory neurons in the nucleus of the solitary tract (NST) project rostrally to the pontine parabrachial nuclei (PBN), which in turn, project to both the thalamic gustatory relay (VPMpc) and the ventral forebrain. In primates, gustatory neurons in NST bypass PBN and project monosynaptically to VPMpc. The primate PBN receives axons from neurons in the visceral afferent areas of medial NST and projects to ventral forebrain. The primate PBN projections to VPMpc, however, are almost non-existent. The differences between rodents and primates are substantial, but the primate data derive only from Old World monkeys (Cercopithecidae, Macaca). In order to examine the central gustatory system in New World monkeys (Ceboidea), horseradish peroxidase conjugated to wheat germ agluttinin (HRP-WGA) was injected into the electrophysiologically identified thalamic taste relay (VPMpc) in 2 marmoset (Callitrix nystax) and 1 squirrel monkey (Saimiri sciureus). An additional squirrel monkey received a similar HRP-WGA injection into the thalamic trigeminal sensory relay (VPM). The VPMpc injections labeled some neurons both in the parabrachial nuclei and in the nucleus of the solitary tract caudal to the entry of the intermediate (facial) nerve. The major concentrations of retrogradely labeled cells, however, connected these 2 cell groups. Unlike rodents, the neurons labeled in the marmoset PBN were concentrated rostrolaterally and were primarily located contralateral to the thalamic injection site. In the squirrel monkey, only a few PBN neurons were labeled on either side. In both New World primate species, neurons were labeled in NST caudal to the intermediate nerve, but compared with Old World monkeys, the distributions were more restricted. At this level, only neurons in the most lateral aspect of the solitary nucleus projected to VPMpc. In addition, a few labeled cells were situated near the anterior 1.5 mm of the solitary tract. In both Old and New World primates species, NST extends rostrally from the level of the intermediate nerve. Dorsal to the oral spinal trigeminal nucleus this anterior extention of NST contained a few labeled neurons. At the caudal border of the principal trigeminal nucleus (PV), the anterior extension expanded into a globular form and was filled with retrogradely labeled neurons. The cytoachitecturally distinct NST extension ended, but labeled neurons continued dorsomedially into the vicinity of the medial parabrachial nucleus. In addition, labeled neurons were evident dorsally and laterally in PV itself. Taken with earlier results in rodents and Old World monkeys, these data imply substantial heterogeneity in the central organization of the gustatory system.

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369.8 CENTRAL GUSTATORY LESIONS I: INTAKE AND TASTE REACTIVITY TESTS. R.Norgren, F.W.Flynn, H.J.Grill, and G.Schwartz.* Dept. Behavioral Science, Col. Medicine, Penn. St. Univ., Hershey and Dept. Psychology, Univ. of Penn., Phila. PA.

In rodents, the gustatory system ascends through three nuclei before reaching its neocortical representation. These areas, the nucleus of the solitary tract (NST), the pontine parabrachial nuclei (PBN), and the parvicellular division of the ventral posteromedial thalamic nulceus (VPMpc), however, each have connections other than those relaying to the next higher level. To begin assessing the functions of these areas, separate groups of rats were prepared with bilateral lesions at each level, and then all the rats were tested for their capacity to exhibit a variety of behaviors guided by gustatory stimuli. Under barbiturate anesthesia, the gustatory nuclei were localized electrophysiologically using tactile, thermal, and sapid tongue stimulation, and cathodal lesions made through the recording electrode (60-80 uA/30 s, N=6 each for NST, PBN, & VPMpc). Full surgical and unoperated control animals (N=4 each) were run in tandem. After a minimum 2 wk recovery, all rats were placed on an 18 h. water deprivation schedule, and given a daily 15 m., 1 bottle consumption test with water and 3 concentrations each of sucrose, NaCl, HCl, and quinine HCl (QHCl). After 2 repetitions of this test, all rats were re-anesthetized and implanted with bilateral intraoral cannulas. After recovery, they were re-tested with the same sapid stimuli infused via the cannulas (1.0 ml in 1.0 m.) and their oro-motor responses recorded on videotape. After the taste reactivity testing, the rats were tested further for sodium appetite, taste aversion conditioning, and a variety of metabolic challenges. The results of these later tests are summarized in accompanying abstracts (Schulkin et al.; Grill et al., this vol.). Although the lesions in each group destroyed much or all of the intended gustatory relay nuclei, neither the thalamic nor medullary damage produced consistent effects in the 15 m., 1 bottle tests. The pontine lesions, however, did significantly lessen the aversion exhibited to the highest concentration of sucrose (1.0 M), NaCl (1.0 M), and QHCl (0.003 M), as well as to the middle concentration of HCl (0.003 M). The taste reactivity test, on the other hand, revealed that NST lesions resulted in significant changes in response to high concentrations of both QHCl and NaCl. Sucrose data remains to be analyzed. Rats with NST lesions exhibited significantly fewer aversive responses than did the control groups or the other rats with lesions. Similar effects were observed with 0.5 M NaCl. Aversive responses to this stimulus were observed in 4/5 PBN, 4/5 VPMpc, and 5/5 control rats, but in none of the 5 NST lesioned animals. The NaCl stimulus also elicited fewer ingestive taste reactivity responses in the NST group. Supported by NIH NS-20397 & AM-21397.

369.9 CENTRAL GUSTATORY LESIONS II: EFFECTS ON SALT APPETITE AND TASTE AVERSION LEARNING. J. Schulkin*, F.W. Flynn, H.J. Grill, & R. Norgren (SPON. L. Hurvich). University of Pennsylvania, & College of Medicine, Pennsylvania State University.

Large central gustatory lesions apparently impair salt appetite as well as conditioned taste aversion. Nevertheless no systematic study has determined the precise gustatory regions that account for these deficits. We tested both of these taste-related behaviors in groups of rats with bilateral lesions in the thalamic taste area (VPMpc, N=6), parabrachial nuclei (PBN, N=5), and nucleus of the solitary tract (NST, N=5), as well as 5 control animals (see Norgren, et al., this vol.). Methods. On day 1, rats were placed on a low NaCl diet and given access to 0.5 M NaCl and distilled water in graduated tubes. On day 2 (when sodium replete) 24 h. fluid intakes were measured and the NaCl solution removed from the home cage. Subsequently, the oro-motor activity of the rats was videotaped while 0.5 M NaCl was infused via an intraoral cannula (2 ml/2 M). Immediately following this taste reactivity test the rats were injected SC with 5 mg of DOCA and 7.5 mg of furosemide. Two h. later an additional SC injection of furosemide was administered to promote additional urinary sodium loss. On day 3 (sodium deplete) the rats were run again in the taste reactivity test using 0.5 M NaCl. Rats were then returned to their home cages and given access to the hypertonic saline for 24 h. On day 4 their 24 h. NaCl intake was measured. Approximately 1 mo. later these groups were examined for their capacity to form a conditioned taste aversion to a novel stimulus (CS=0.3 M L-alanine; US=1.5 mEq LiCl IP) using the taste reactivity protocol. Results. While Na replete, intraoral infusions of 0.5 M elicited fewer aversive responses from the 3 lesion groups than from the controls, although only the NST group reached statistical significance; there were also no differences in their NaCl home cage ingestion. When tested following salt appetite arousal, both control and VPMpc groups produced significantly greater ingestive responses in the taste reactivity test than when they were Na replete and increased their home cage intake of NaCl ($p's < .05$). The PBN and NST groups neither changed their taste reactivity response nor their 24 h. NaCl ingestion. In the taste aversion conditioning test, VPMpc and NTS groups were similar to control rats in significantly decreasing their ingestive responses and increasing their aversive responses as a function of the number of daily taste-LiCl pairings. The PBN group was significantly different from the other groups; 2 of the 4 PBN rats tested failed to display taste aversion learning. The salt appetite results suggest that first and second order brainstem gustatory regions contribute to the neural processing of salt appetite. Future research will specify the nature of the deficits in salt appetite in the NTS and PBN groups. Supported by NIH AM-21397, NS-20397, NS-21833 & MacArthur Postdoc.

369.10 CENTRAL GUSTATORY LESIONS: III. REGULATION OF ENERGY BALANCE. H.J. Grill, F.W. Flynn and R. Norgren. Dept. Psychol., & Inst. Neurol. Sci., Univ. of Pennsylvania, Philadelphia and Dept. Behav. Sci., College of Med., Penn. State Univ., Hershey, PA

Behavioral and autonomic responses act in concert to control energy balance. The experiments reported here examine the effects of central gustatory lesions on these energy balance responses. The lesion procedure is described in the accompanying abstract (Norgren et al). Rats had bilateral lesions in the nucleus of the solitary tract (NST, n=5), parabrachial nuclei (PBN, n=4), or gustatory thalamic area (VPMpc, n=5). Seven control rats were tested as well. Rats had ad libitum access to powdered chow and water. The following sequence of tests occurred approximately 2 mo. post-lesion; a minimum of 3 days separated each test. Method (a) 2DG and insulin-elicited feeding. Chow was removed at 0800 h. and 1 h. later rats were given IP injections of either saline (0.15M), 250 mg/kg 2DG, or 2 U/kg regular insulin. Food dishes were then filled with fresh chow, weighed, and returned to the rats' cages. Four hours later, the dishes (corrected for spillage) were re-weighed. (b) Intragastric loads. Rats were food deprived for 24 h. Prior to the return of the chow, rats were intubated 20 ml/kg saline (0.15M) or sweetened condensed milk/water solution (2 kcal/ml). Food dishes were reweighed 1 and 3 h. later. (c) Hyperglycemic response. Food was removed and 1 h. later a blood sample taken. The rats were then injected IP 250 mg/kg 2DG, and blood samples taken 1 and 4 h. later. Results All 4 groups consumed significantly more chow following 2DG or insulin than following saline injection ($p's<.05$). In response to saline intubation, rats of the control, NTS, PBN, and VPMpc groups consumed similar amounts of chow. Food intake was significantly reduced in control rats and rats with NTS and VPMpc lesions at 1 h. (median 85%) and 3 hr (median 60%) following the diet intubation. Diet significantly reduced chow intake by rats with PBN lesions, but the suppression at 1 h. (73%) and 3 h. (34%) was significantly less than that seen in control rats. Plasma analysis revealed that glucose levels of the 4 groups were similar prior to the 2DG injection. The hyperglycemic response elicited by 2DG was of comparable magnitude in the groups at 1h. (240-300 mg/dl) and 4 By 4 h., plasma glucose levels had decreased, but remained significantly greater than pre-injection levels for the 4 group In summary, gustatory nuclei lesions did not affect the ingesti of a familiar food source following the metabolic challenge pos by 2DG and insulin treatments. The stomach load test, however, indicated that the PBN is involved in the caudal brainstem circuitry mediating the effects of energy repletion signals generated by stomach loads on food intake. Whether this effect i due to the damage of ascending vagal projections or the integrat of taste and postingestional feedback remains to be determined. Supported by NIH AM 21397 & NS 20397

## BRAIN METABOLISM IV

370.1 PHYSOSTIGMINE-INDUCED ALTERATIONS OF LOCAL CEREBRAL GLUCOSE UTILIZATION IN AWAKE RATS. H.W. Holloway*, T.T. Soncrant*, D.M. Larson* and S.I. Rapoport (Spon: P. Newhouse). Laboratory of Neurosciences, National Institute on Aging, National Institutes of Health, Bethesda, MD 20205.

Physostigmine (PHYSO) is a drug that enhances cholinergic neurotransmission in the central nervous system and the periphery by inhibiting the acetylcholine-degrading enzyme, acetylcholinesterase. PHYSO reportedly improves short-term memory in experimental animals (Cox et al., Neuropharmacol., 13: 205, 1975) and in human subjects (Christie et al., J. Psychiat., 138: 46, 1981). This action has been ascribed to an increased effect of acetylcholine at CNS sites, and suggested a potential therapeutic role for PHYSO in Alzheimer's disease, in which large cholinergic deficits occur. We wished to elucidate the regional CNS metabolic changes produced by PHYSO and to relate them to the patterns of metabolic change that we have reported after arecoline, a muscarinic agonist (Soncrant et al., Brain Research, in press) and nicotine (McNamara et al., Trans. Am. Soc. Neurochem., 16:149, 1985).

Groups of 5-6 male, 3 mo old, awake Fischer-344 rats received physostigmine sulfate 2 mg/kg or vehicle i.p. To reduce autonomic effects of PHYSO, all rats, except those of one control group, were pretreated with methylatropine and hexamethonium, muscarinic and nicotinic antagonists, respectively, that do not enter the brain. The behavioral effects of PHYSO were scored periodically on a scale of 0 (no behavioral change) to 4 (extreme tremor) for 2 h after its administration. Local cerebral glucose utilization (LCGU) was measured by the quantitative, autoradiographic [14C]-deoxyglucose method (Sokoloff et al., J. Neurochem. 28: 897, 1977), beginning at 10, 30, or 60 min after PHYSO administration.

Tremor was observed in all animals that received PHYSO. It peaked at 10 min, and largely subsided by 30 min. LCGU measured during peak tremor activity (10 min) was increased significantly ($p < 0.05$) in 68 of the 86 brain areas examined. No areas of decreased LCGU were found. LCGU effects of PHYSO were smaller at later time points and were related to the intensity of behavior observed. The pattern of the brain metabolic response to PHYSO is a composite of those produced by arecoline and nicotine. Increased LCGU was observed in neocortical layers IV and V and in the hippocampus, where arecoline, but not nicotine, elevated LCGU. PHYSO also increased LCGU in the subiculum, terminal nucleus of the optic tract, and the substantia nigra, in which LCGU rose after nicotine, but not after arecoline.

These preliminary findings demonstrate that PHYSO increases regional CNS metabolic activity by enhancing cholinergic neurotransmission at both muscarinic and nicotinic receptors.

370.2 ALTERATIONS IN REGIONAL BRAIN METABOLISM FOLLOWING CHRONIC HALOPERIDOL TREATMENT IN RATS DETERMINED BY THE $C^{14}$-DEOXYGLUCOSE METHOD. J.M. FREY, R.D. HUFFMAN, R.D. BELL*. Dept. of Pharmacology, Div. of Neuropharmacology, and Dept. of Medicine, Div. of Neurology, Univ. of Texas Health Science Center, San Antonio, Texas 78284.

Acute administration of the dopamine receptor antagonist haloperidol has previously been reported to produce regional changes in glucose utilization (ℓ-CMRg) within extrapyramidal, mesolimbic and cortical areas (McCulloch et al., Brain Res., 243:81-90, 1982). Since it has also been reported that the cataleptogenic action of haloperidol is attenuated upon repeated administration in rats and that tolerance develops to the extrapyramidal side-effects but not the neuroleptic action of haloperidol in humans (Lloyd & Worms, Adv. Biochem. Psychopharmacol., 24:253-258, 1980; Ayd, F.J. Intern. Drug. Ther. Newslett., 6:33-56, 1971) we thought it would be of interest to see whether the acute effects of haloperidol on regional brain metabolism persisted after prolonged administration.

Male Sprague Dawley rats were administered haloperidol (0.01-.02%) in their feed for at least 30 days. Control rats received plain chow. Under ketamine anesthesia, these animals were instrumented with femoral arterial and venous catheters and allowed to recover a minimum of 3 hours, after which a quantitative 2-deoxyglucose (2-DG) study was performed as described by Sokaloff et al, J. Neurochem., 28:897-916, 1977). Each animal was injected with an intravenous bolus of $^{14}$C-2-DG (8 µci/100gms). Arterial blood samples (0.2cc) were drawn at timed intervals up to 45 minutes to determine arterial 2-DG and glucose concentration curves. At 45 minutes, animals were sacrificed, the brain removed and frozen in liquid nitrogen. The tissue was then cut into 20µm sections and exposed to x-ray film (Kodak, SB-5) for 2 weeks. $^{14}$C tissue concentrations in structures of interest were obtained with the use of a Gamma Scientific microdensitometer. Significant decreases in glucose utilization ($p<.01$) were found within cortex, thalamus (VL,VM,DM), entopeduncular nucleus, hippocampus, amygdala, and superior colliculus as well as in the caudate-putamen and subthalamic nucleus ($p<.05$). Increases in glucose metabolism were detected within the nucleus accumbens ($p<.01$) and the nucleus pedunculopontis ($p<.05$). Whereas McCulloch et al., 1982 reported dose-related changes in glucose utilization within substantia nigra and globus pallidus after acute haloperidol, we found no alterations in ℓ-CMRg within those structures after 30 days of chronic treatment.

370.3 THE EFFECT OF TYPICAL AND ATYPICAL NEUROLEPTIC AGENTS ON REGIONAL CEREBRAL GLUCOSE METABOLISM IN THE RAT. P.M. Garvey, A. Braun*, L.C. Kao and H.L. Klawans.* Dept. of Neurological Sciences, Rush-Presbyterian St. Lukes Med. Ctr., Chicago, Il. 60612.

Exposure to typical neuroleptic agents such as haloperidol (Hal), chlorpromazine (Cpz) or fluphenazine (Flu) produces a number of acute side effects such as parkinsonian symptoms and dystonias commonly referred to as extrapyramidal side effects (EPSE). Neuroleptic agents such as thioridazine (Thio) or clozapine (Cloz) possess antimuscarinic activity in addition to their action at DA receptors and are less associated with these effects. As such, they are referred to as atypical. We examined the effect these agents have on glucose metabolism in the freely responding rat utilizing the 2 deoxyglucose method.

All neuroleptics studied reduced metabolic activity in the anteromedial frontal cortex and the cingulate cortex while increasing activity in the lateral habenula. Typical neuroleptics however were least effective in reducing anteromedial frontal activity and most effective in elevating activity in the lat. habenula. Typical neuroleptics elevated activity in the caudal regions of the striatum as well as the globus pallidum whereas Thio and Cloz had no affect or actually reduced activity respectively. A similar trend was also observed in the ventral tier of the thalamus. On the other hand atypical neuroleptics increased activity in all regions of the CNS known to possess choline acetyl transferase activity such as the hippocampus, the septal region, the amygdala, n. diagonal band, the lateral preoptic area and especially the ventral pallidum. These data suggest that although there are common effects, typical and atypical neuroleptics can influence metabolic activity differentially which may explain their propensity to induce EPSE's. In this regard, discriminant analysis performed on both neuroleptic groups and saline treated controls demonstrated that the anteromedial frontal, the cingulate and the suprarhinal cortices as well as the caudal striatum, the ventral thalamus and the superior colliculus could segregate neuroleptic effects with high confidence. This would suggest that the 2 deoxyglucose method could model and potentially predict neuroleptic activity.

370.4 THE EFFECTS OF SEROTONERGIC AGENTS QUIPAZINE AND METHIOTHEPIN ON LOCAL CEREBRAL GLUCOSE UTILIZATION IN THE RAT. G.L. Ricchieri*, T. T. Soncrant*, S.I. Rapoport (SPON: C. Grady). Laboratory of Neurosciences, National Institute on Aging, National Institutes of Health, 10/6C103, Bethesda, MD 20205.

Quipazine (QUIP) is a relatively specific agonist at serotonin (5-hydroxytryptamine, 5-HT) receptors. QUIP produces stereotyped behavior in rats similar to that seen after the administration of L-tryptophan or of 5-hydroxytryptophan (5-HT precursors). This behavioral "5-HT syndrome" includes hyperactivity, tremor, head shaking and weaving, hindlimb abduction, backing, circling and shivering. Methiothepin (METH), a 5-HT receptor antagonist, causes catalepsy characterized by reduced motor activity in rats.

We measured local cerebral glucose utilization (LCGU), using the quantitative [14C]-2-deoxy-D-glucose method, in awake 3 mo Fischer rats at 1 h after administration of QUIP maleate 20 mg/kg or saline i.p., and at 1, 2, 3 or 4 h after METH maleate 0.1 mg/kg or vehicle, i.p. Behaviors were rated subjectively at fixed time intervals after QUIP and METH.

Intense behavior (5-HT syndrome) was seen in all animals at 1 h after QUIP. METH produced catalepsy that peaked at 3 h after drug treatment. LCGU was increased significantly ($p<0.05$) at 1 h after QUIP in 48% of the 92 brain regions examined. The mean increase in LCGU was 20-25%. LCGU rose in olfactory regions, limbic areas (dentate gyrus of ventral hippocampus, mammillary nuclei, medial amygdala), in most thalamic and subthalamic nuclei examined, raphe nuclei, and in regions without a blood-brain barrier (area postrema, subfornical organ). QUIP increased LCGU also in several extrapyramidal motor regions (caudate n., globus pallidus, substantia nigra, subthalamic n., inferior olive, cerebellum) which were metabolically activated also during stereotypic behavior produced by bromocriptine, a dopamine agonist (Pizzolato et al., Brain Res., in press). In contrast, LCGU was reduced in 35% of regions examined at 3 h after METH administration. The basal ganglia and cortical, hippocampal, and thalamic regions were affected by METH.

These results indicate that pharmacological activation of central 5-HT pathways can produce widespread increases in brain functional activity, by direct or indirect mechanisms, and cast doubt upon a net inhibitory role for 5-HT in the CNS. Activation of a behavior-related motor pathway by both QUIP and bromocriptine suggests that the functional effects of 5-HT activity can be mediated via dopamine pathways, in accord with biochemical findings (Chase, 1974; Dray, 1980). Finally, reductions in LCGU after METH are inconsistent with the notion that METH primarily antagonizes presynaptic 5-HT receptors (Pettibone, 1984). Instead, they suggest an important role for METH at postsynaptic 5-HT receptors.

370.5 REGIONAL CEREBRAL GLUCOSE UTILIZATION DURING KETAMINE ANESTHESIA. D.W. Davis*, A.M. Mans*, J.F. Biebuyck* and R.A. Hawkins (SPON: W.P. Graham). Departments of Anesthesia and Physiology, M.S. Hershey Medical Center, The Pennsylvania State University College of Medicine, Hershey, PA 17033

The anesthetic ketamine appears to produce both excitatory and depressant effects in the brain. Reports on its effects on cerebral glucose use ($CMR_{Glc}$) are not clear (1,2,3). We measured regional $CMR_{Glc}$ in unrestrained rats given 2 different doses of ketamine. The rats had catheters placed in the right atrium 2 days before the experiment, and were housed in chambers insulated against outside stimuli until completion of the experiment. This technique enabled infusion and blood withdrawal without apparent awareness by the rat. Ketamine was given as an initial bolus followed by a 20-minute infusion to saturate all compartments. This ensured a steady level of ketamine throughout the 5-minute experimental period, following the infusion. A total of either 5 mg/kg or 30 mg/kg was given. Regional $CMR_{Glc}$ was measured using $^{14}$C-glucose and autoradiography. Rats given ketamine were compared to saline-infused rats. The 2 doses of ketamine produced strikingly different behavior. Rats given 30 mg/kg remained motionless throughout the entire period whereas those given 5 mg/kg moved continuously about the cage. Cerebral metabolism was likewise affected differently by the 2 doses of ketamine. Rats receiving 5 mg/kg showed elevated rates of $CMR_{Glc}$ (13-43%) in many (26 of 43) brain areas, compared to control. The greatest increases were seen in several cortical areas, the posterior hippocampus and white matter areas. In rats receiving 30 mg/kg a different pattern was observed; 3 areas, the inferior colliculus, vestibular nucleus and inferior olive showed significant decreases of 14 to 29%. No other areas were affected at this dose. These results may explain the conflicting earlier reports on the effects of ketamine. Hawkins et al. (1), using $^{14}$C-glucose after a bolus of 35 mg/kg ketamine, found essentially no change in $CMR_{Glc}$. In contrast, others report increases in some areas and decreases in others (2,3). In the latter two studies $CMR_{Glc}$ was measured using the deoxyglucose method which requires an experimental period of 45 minutes. During this time the ketamine concentrations and therefore the state of anesthesia would be expected to change; the results would reflect a combination of anesthetic and recovery states. Our results obtained during 5 minutes under steady-state conditions show that the effect of ketamine on both behavior and cerebral metabolism is dose-dependent, and that specific brain areas are affected differently.

1. Hawkins RA, Hass WK, Ransohoff J. Stroke 10:690-703, 1979
2. Crosby G, Crane AM, Sokoloff L. Anesthesiol. 56:437-443, 1982
3. Nelson SR, Howard RB, Cross RS, Samson F. Anesthesiol. 52:-330-334, 1980

370.6 EFFECT OF CHRONIC NALTREXONE ON REGIONAL CEREBRAL OXYGEN CONSUMPTION IN THE CAT. E. Buchweitz* and H.R. Weiss*, (SPON. B. Hine) UMDNJ-Rutgers Medical School, Heart and Brain Circulation Laboratory, Department of Physiology and Biophysics, Piscataway, NJ 08854

The purpose of this investigation was to correlate the effects of up-regulation of opiate receptors by chronic naltrexone administration on average and regional cerebral blood flow, oxygen extraction and oxygen consumption. Fourteen adult mongrel cats were administered subcutaneous injections of 1 mg/kg naltrexone HCl or 1 ml 0.9% saline twice daily for 21 days. Two days later, regional cerebral blood flow was monitored using radioactively tagged microspheres. The animals were sacrificed and prepared for microspectrophotometric analysis of regional cerebral venous and arterial oxygen saturation. Cerebral oxygen consumption was calculated as the product of cerebral blood flow and oxygen extraction. Chronic naltrexone did not significantly alter blood pressure, heart rate or blood gas parameters. Average cerebral blood flow increased significantly from 47.9±3.4 (mean±S.E.M.) ml/min/100g to 80.3±6.5 ml/min/100g in the chronic naltrexone treated group. Flow was significantly increased in the cortex, lenticulate nuclei, cerebellum, thalamus and pons. Average oxygen consumption was not significantly altered by treatment. The distribution of flow and oxygen consumption among the examined regions was significantly altered in the animals receiving chronic naltrexone injections. Flow was significantly higher in the lenticulate nuclei, and significantly lower in the hypothalamus of the treated group. Chronic opiate receptor blockade significantly increased average cerebral blood flow, while not altering average cerebral oxygen consumption. These changes were not restricted to brain regions dense in neuronal opiate receptors. Since cerebral blood flow was significantly increased without a concommittant change in oxygen consumption, chronic naltrexone administration may have a direct effect on the cerebral vasculature.

370.7 REGIONAL CEREBRAL BLOOD FLOW FOLLOWING INTRAVENOUS HEROIN IN RATS. T.C. Trusk and E.A. Stein. Department of Biology, Marquette University, Milwaukee, WI 53233.

Morphine and other opiates are known to generally decrease cerebral use of oxygen and glucose in a naloxone-reversible manner. The purpose of this work is to observe the effects of heroin on blood flow in discrete areas of the rat brain. A bolus of 0.2 mg/kg heroin or saline vehicle was injected into the femoral vein of conscious, restrained rats. One minute later iodo($^{14}$C)antipyrine in saline (6 µCi/100 g) was infused for 30 seconds into the same femoral vein. Femoral artery blood was sampled every 5 seconds during the infusion. The rat was then immediately decapitated, the brain rapidly removed and frozen in isopentane. Each brain was cut into 20 µm sections which were dried onto glass slides and exposed to x-ray film for 5 days. The optical density of selected brain regions was determined with a Spatial Data Image Analyzer and converted to regional cerebral blood flow (RCBF) by means of the Kety-Schmidt equation. Heroin produced differential effects on RCBF in relation to saline-injected rats. Significant increases in blood flow (15-40%) were observed in somatomotor cortex, dorsal and ventral striatum, hippocampus, and corpus callosum. RCBF significantly decreased 15-20% in frontal cortex, globus pallidus, and the lateral hypothalamus. RCBF remained unchanged in the other brain regions studied. It has been suggested that RCBF varies directly to the local metabolic demands of functional activity. Previous observation of local cerebral glucose utilization 30-45 minutes following morphine or opioid peptide injection reported global decreases in brain activity. The present results suggest that heroin may not only decrease brain activity but may also increase functional activity in selected regions shortly after systemic injection. These changes may represent a neural substrate of the behaviorally-excitant effects observed immediately after opiate injections. Supported in part by NIDA grant #DA02234 to EAS.

370.8 THE EFFECTS OF CHRONIC DIPHENYLHYDANTOIN ADMINISTRATION ON CEREBRAL METABOLISM IN THE ADULT RAT. J.W. Melisi*, D.L. Dow-Edwards, R. Barbour* and T.H. Milhorat. Laboratory of Cerebral Metabolism, Department of Neurosurgery, SUNY Downstate Medical Center, Brooklyn, N.Y. 11203.

Diphenylhydantoin (DPH) is one of the most widely used anticonvulsant agents in humans. In the central nervous system, it exerts a protective effect by altering the seizure threshold. Presumably, then, a drug such as this should have profound effects on cerebral metabolic activity. To test this, we employed the quantitative autoradiographic $^{14}$C deoxyglucose method to measure rates of in vivo glucose utilization in the rat brain (Sokoloff et al, J Neurochemistry 28:897-916,1977). We analyzed 31 structures in the adult rat brain to determine if DPH has global or site-specific effects on cerebral metabolism.

Male Sprague-Dawley rats (350-400g) were divided into two groups. The experimental group received a loading dose of 100 mg/kg DPH ip (Dilantin, Parke-Davis) in two doses given eight hours apart. For the next five days, 50 mg/kg DPH ip per day was administered. Control animals received equivalent volumes of vehicle kindly supplied by Parke-Davis. On the seventh day, the rats were anesthetized with Halothane and arterial and venous femoral catheters were placed. The rats were allowed to recover over the next two hours. Thirty minutes prior to deoxyglucose administration, the final dose of drug or vehicle was given. $^{14}$C labeled deoxyglucose (125 uCi/kg) was then given as a bolus through the venous catheter. Timed arterial samples were taken over the next 45 minutes and analyzed for glucose and $^{14}$C deoxyglucose concentrations. The animals were sacrificed after 45 minutes, the brains rapidly removed and frozen in isopentane chilled to -35°C. The brains were sectioned for autoradiography as described by Sokoloff et al(1977).

Blood samples taken during the deoxyglucose procedure were analyzed for levels of Dilantin using flourometric methods. Experimental animals had average plasma drug levels of 10.7ug/ml, similar to that found to be therapeutic in humans. Significant differences in local cerebral glucose utilization were found in 21 of 31 structures studied. All of the differences represented decreases in glucose utilization in treated animals compared to controls. The largest changes were noted in the auditory pathways, hippocampus, thalamus, basal ganglia, fastigial and lateral septal nuclei and throughout the cortex. These data support previous studies that show DPH affects multiple sites in brain. It also indicates that an increase in seizure threshold, reported by others to be due to an increased duration and decreased amplitude of the action potential, results in a decrease in metabolic rate.

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370.9 CHANGES IN BRAIN $pO_2$ DURING SOMAN-INDUCED SEIZURES IN THE RAT. T.J. Lynch*[1], C.S. Stratton*[2] and J.F. Glenn[2]. [1]Dept. of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, D.C., 20307-5100, and [2]Neurotoxicology Branch, US Army Medical Research Institute of Chemical Defense, APG, MD 21010-5425.

CNS neuropathies and axonopathies have been demonstrated in animals exposed to either convulsive (McLeod et al., Neurotoxicol. 5:53, '84) or subconvulsive (Petras, Fundam. Appl. Toxicol., 1:242, '81) doses of the irreversible anticholinesterase soman. Since brain $pO_2$ has been shown to drop repeatedly during seizures induced by the bicyclic organophosphates (Lynch et al., Neurosci. Abs., 10:123.10, '84), the potential role of hypoxia as an etiological factor in development of soman-induced brain damage was investigated in free-ranging rats. Following injection of a convulsive dose of soman (100-120 mcg/Kg, s.c.), brain $pO_2$ levels were measured polarographically from platinum electrodes implanted in various limbic sites, with concurrent monitoring of behavioral signs and neocortical EEG.

Brain $pO_2$ was found to increase by as much as 100% during the early ictal stages. Behavioral signs during this period included forelimb clonus, excessive grooming, postural abnormalities and intermittent myoclonus. The development of typical physiological signs of anticholinesterase toxicity, such as excessive salivation, paralleled increases in paroxysmal EEG activity. As the EEG and behavioral signs of seizure intensified, the $pO_2$ levels were maintained above control except for transient sharp drops by as much as 56% during periods when whole body tonus prevented adequate ventilation. An increase in the variability of the normal $pO_2$ oscillations from 2-7% to 9-24% of baseline was associated with increased fluid secretions. Fluid asphyxiation appeared to be the proximate cause of a final slow decline in $pO_2$ since respiratory frequency was increased during all but the immediate pre-mortem period. No $pO_2$ decline occurred in the absence of marked secretions.

Hypoxia does not appear to be a major cause of soman-induced brain damage. Rather than a lack of adequate tissue oxygenation, excitotoxicity or mismatches between increased metabolic demand during soman-induced seizures (McDonough et al., Neurotoxicol., 4:203,'84) and metabolic capacity would appear to be more likely contributing factors to the neuronal damage observed by others.

370.10 DIFFERENCES AMONG METABOLIC CONVULSANTS. J. Wright*, D. Martin*, Y. Walker*, A. Borders*, L. Watson*, P. Rauch*, M. Liveriatos*, L. DiCarlo*, D. Ryan*, J.A. Brown* and Mary Ann Marrazzi (SPON: J.A. Benjamins). Dept. Pharmacology, Wayne State University School of Medicine, Detroit, Mich. 48201

Insulin hypoglycemia, fluoroacetate and 2-deoxyglucose (2DG) all induce metabolic convulsions, presumably due to a fuel shortage. Insulin hypoglycemia decreases overall glucose metabolism by decreasing the glucose available to brain. Fluoroacetate blocks the Krebs cycle, by being converted to fluorocitrate which blocks the citrate to isocitrate step. 2DG blocks glycolysis at the hexose isomerase step immediately after hexokinase. These three metabolic convulsants can be quantitatively differentiated on the basis of the effects of gold thioglucose (GTG) and 5-thioglucose (5TG) on the convulsive sensitivity in female CBA/J mice. A single IP injection of GTG causes a cytotoxic lesion focused in the ventromedial-arcuate hypothalamus (VMH) and a biphasic change in the sensitivity to insulin hypoglycemic convulsions. The percent convulsions in a population is decreased at early times (16-24 hours) and increased at later times (1-2 weeks) after GTG. Blood glucose measurements indicate that both changes are differences in the convulsive response to equal hypoglycemia, rather than the hypoglycemic response to insulin. Non-metabolic Metrazol induced convulsions are not affected, indicating that the generalized brain excitability is not altered. 5TG simulates the early component of the GTG effect on insulin hypoglycemic convulsions, but has no effect at 1 week. It also does so without causing the VMH lesion. In contrast to insulin hypoglycemic convulsions, the sensitivity to either fluoroacetate or 2DG induced convulsions is not affected by GTG or 5TG. Therefore, these three metabolic convulsants are qualitatively, as well as quantitatively, different. The GTG phenomenon also suggests a regulatory center involved in adjusting the brain's convulsive response to insulin hypoglycemia - a "GTG lesioned glucostat" (J. Pharmacol. Exper. Therap. 219:258, 1981). The 2DG and fluoroacetate data suggest that the portions of glucose metabolism blocked by these convulsants are not necessary for the operation of the proposed "GTG lesioned glucostat".
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370.11 DIAZEPAM PREVENTS ORGANOPHOSPHATE-INDUCED INCREASES IN BRAIN CHOLINE LEVELS. C. J. Flynn and L. Wecker. Louisiana State University Medical Center, New Orleans, LA 70112.

The organophosphate cholinesterase inhibitors sarin and soman induce convulsions in rats when administered at doses that inhibit acetylcholinesterase activity by 90-100%. When measured 1-2 hours after the onset of convulsions, levels of free choline in brain are significantly increased. Since this increase in free choline may result from phospholipid breakdown, which has been shown to occur during convulsions induced by other agents, it was of interest to determine whether pretreatment of rats with diazepam would prevent the convulsions and concomitant increase in choline levels. Male Sprague-Dawley rats were injected with either saline or diazepam (4 mg/kg, i.p.) 30 minutes prior to the administration of saline, soman (57-64 ug/kg, s.c.), or sarin (95-100 ug/kg, s.c.), doses of the organophosphates that inhibit acetylcholinesterase activity in striatum and hippocampus by 90-100%. Animals were killed two hours after the second injection by head-focused microwave irradiation and the levels of choline in striatum and hippocampus were quantified by pyrolysis gas chromatography. When rats were injected with diazepam prior to the administration of sarin or soman, no seizures were evident, whereas in rats injected with saline prior to sarin or soman, seizures were exhibited by all rats. Two hours after the administration of sarin or soman, choline levels in striatum increased significantly from control values of 33 nmoles/g to 46 nmoles/g and 54 nmoles/g, respectively. Diazepam pretreatment completely prevented the organophosphate induced increase, and choline levels after the combined injections were not different from control values. In the hippocampus, choline levels increased from 23 nmoles/g to 62 nmoles/g and 68 nmoles/g following the administration of sarin and soman, respectively. Diazepam pretreatment partially prevented this increase and levels of choline were only 38 nmoles/g and 42 nmoles/g following the administration of sarin and soman, respectively. Diazepam by itself had no effect on free choline levels in either area. These results indicate that the convulsions induced by these two organophosphates cause elevations in the levels of free choline in brain which are prevented by pretreatment with diazepam. It is likely that soman and sarin enhance the release of choline from phospholipids, a finding consistent with studies indicating a relationship between seizure activity and phospholipid hydrolysis. (Supported by USAMRDC #DAMD17-83-C-3012).

370.12 2-(1-$^{14}$C)-DEOXY-D-GLUCOSE UPTAKE IN DISCRETE AREAS OF BRAIN IN DIFFERENTIALLY HOUSED MICE. S. McCall, C. VanderWende, and M.T. Spoerlein. Rutgers University, P.O. Box 789, Piscataway, New Jersey 08854.

2-(1-$^{14}$C)-deoxy-D-glucose (2-d-g) has been shown to be transported by the glucose transport system into neurons where it is phosphorylated and accumulates because it can not be further metabolized. This property of 2-d-g has served as a means of measuring neuronal activity since an increase or decrease of neuronal activity will lead to a respective increase or decrease of 2-d-g uptake and accumulation.

Individual housing (social isolation) of male mice produces marked behavioral and neurochemical changes. Some of the most prominent behavioral changes are the development of fighting between conspecific males and a hyperactivity syndrome. We were interested in determining whether there is an increase of activity in certain areas of the brain related to motor and behavioral function using the accumulation of 2-d-g.

Group housed CF-1 male mice, 35 days of age, served as the zero time control animals. Other animals were either group housed or individually housed for 1, 2, 3, or 4 weeks. On the day of the test, 150 uCi were administered i.v. and the mice sacrificed by decapitation 45 minutes later. The brains were rapidly removed, weighed, and dissected over ice to remove the striatum, hippocampus, hypothalamus and frontal cortex. Each area was dissolved in 0.4 ml Protosol to which was added 4 ml of Aquasol II. Radioactivity was measured in a liquid scintillation spectrometer and the results expressed as DPM/mg of tissue. Results show in all areas studied an increased accumulation of 2-d-g with increasing time of isolation.

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370.13 **THE EFFECTS OF OCTANOIC ACID ON DOPAMINE, SEROTONIN AND THEIR METABOLITES IN THE CAUDATE NUCLEUS AND HYPOTHALAMUS.** **C.S. Kim*, C.R. Roe*, J.D. Mann and G.R. Breese. Dept. of Neurology, Psychiatry, Biol. Sci. Res. Ctr., Univ. of North Carolina Sch. of Med., Chapel Hill, NC 27514 and Dept. of Pediatrics, Duke Univ. Med. Ctr., Durham, NC 27709.**

**The relationship between the encephalopathy of medium-chain acyl-CoA dehydrogenase deficiency (MCAD) and elevation in serum octanoic acid is not well understood. Accumulation of endogenously produced organic acids in CSF and brain, secondary to reduced clearance by choroid plexus (CP), could be a contributing factor in the development of encephalopathy. The present in vivo study was undertaken to determine whether organic acid metabolites of monoamines are accumulated in the brain after treatment of octanoic acid. Male Sprague-Dawley rats (100-150g) were injected i.p. with either saline or octanoic acid (500 or 1000 mg/kg). Two hours later, rats were sacrificed by decapitation and their brains rapidly removed. The caudate nucleus (CN) and hypothalamus (H) were dissected and immediately frozen. Dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), 3-methoxy-4-hydroxyphenylacetic acid (HVA), serotonin and 5-hydroxy-indoleacetic acid (HIAA) were determined in these tissues by HPLC. In the controls, the concentrations in CN and H for HVA were 6.47 + 0.51 and 0.13 + 0.06 ng/mg protein and for HIAA were 4.59 + 0.17 and 7.03 + 0.24 ng/mg protein, respectively. Octanoic acid (1000 mg/kg) elevated HVA by 54% ($p < 0.05$) in CN and 338% ($p < 0.05$) in H, while increasing HIAA in both CN and H by approximately 50% ($p < 0.05$) compared to control. A lower dose (500 mg/kg) increased HIAA by 29% in CN ($p < 0.05$) and 20% in H ($p < 0.05$). Treatment with octanoic acid failed to change the level of dopamine, serotonin and DOPAC in CN and H.**

**The present study demonstrates that octanoic acid produces major changes in the level of endogenous organic acid metabolites of HVA and HIAA in CN and H. We have previously shown that octanoic acid disrupts the mitochondrial ultrastructure in CP and inhibits the organic anion transport system (Kim et. al., Brain Res. 310:149-153, 1984). Since this transport system in the CP is responsible for the excretion of monoamines metabolites from brain to plasma, the present study provides further evidence that octanoic acid disrupts transport of acid compounds. Failure to clear organic acid metabolites from CSF by CP could contribute to the development of encephalopathy in children with MCAD who have elevated levels of octanoic acid systemically.** **This study was supported in part by NIH grants: HD-03110 and ES-03458.**

370.14 BRAIN GLUCOSE USE, MONOAMINES AND AMINO ACIDS AFTER NUTRITIONAL THERAPY OF HEPATIC ENCEPHALOPATHY. A.M. Mans*, D.W. Davis*, J.F. Biebuyck* and R.A. Hawkins. Departments of Anesthesia* and Physiology, M.S. Hershey Medical Center, The Pennsylvania State University College of Medicine, Hershey, PA 17033

Portacaval shunting in rats produces several metabolic abnormalities, including a 25-30% depression of brain glucose use. Plasma and brain amino acid concentrations are altered and there may be an imbalance in brain monoamine neurotransmitters. With the idea that the latter may promote encephalopathy, treatments have been devised to try to normalize the brain amino acids which are precursors for monoamine synthesis. Thus, amino acid mixtures rich in branched chain amino acids, which compete with the precursor amino acids for transport into brain, have been infused into patients with hepatic encephalopathy. In this study we gave oral glucose or glucose with valine, leucine and isoleucine for two weeks to shunted rats. The diet was made up in agar blocks containing a salt mixture to prevent mineral loss. No other food was provided; the rats had free access to water. Treated rats were compared with normally fed rats. Brain glucose use was measured using $^{14}$C-glucose and autoradiography in 8-minute experiments. One brain hemisphere was used for autoradiography and the other for measurement of amino acids and the monoamines norepinephrine, dopamine, serotonin, and 5-hydroxyindoleacetic acid (5-HIAA). Shunting produced the typical pattern in plasma of raised glutamine, phenylalanine, tyrosine and histidine, and lowered branched chain amino acids and threonine. The elevated amino acids were lowered by both types of treatment; the branched chain amino acids were further depressed by glucose alone but in the normal range in the group given amino acids. In brain, the monoamine precursor amino acids tryptophan, tyrosine and phenylalanine were increased after shunting. Both treatments lowered phenylalanine and tyrosine; glucose alone normalized tryptophan. The high glutamine was normalized by both treatments. Norepinephrine was significantly higher after shunting and unaffected by treatment, even though both plasma and brain phenylalanine and tyrosine were lowered. Dopamine was normal in all groups. Brain serotonin and 5-HIAA were significantly raised after shunting. The glucose diet brought both into the normal range but glucose with amino acids had less effect. Thus, several metabolic abnormalities believed to be important in the development of hepatic encephalopathy were reversed by nutritional therapy. In contrast, neither treatment had any effect on brain glucose use, which remained depressed in all 40 brain areas examined. Therefore, since function appeared unimproved, these results raise the question of whether changes in brain amino acids and monoamines are of etiologic significance in hepatic encephalopathy.

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370.15 ALTERED CEREBRAL AND HEPATIC PROTEIN AND AMINO ACID METABOLISM IN AN ANIMAL MODEL OF A REYE-LIKE SYNDROME. P.H. Schwartz, C.G. Wasterlain, B.E. Dwyer, and E.L. Arcinue Dept. Neuroscience, U.C.L.A. Sch. of Med., Los Angeles, CA 90024

Margosa oil (MO, an extract of the seeds of the neem tree in Indomalaysia) has been shown to give rise to a Reye-like syndrome (RLS) in human children. The purified oil has been used as a panacea for millenia but the crude oil has been shown to be toxic. Using the toxic oil we have developed an animal model of this RLS and now report the results of a dose-response study of the effects of MO on cerebral and hepatic protein and amino acid metabolism. The data show that early abnormalities in hepatic protein synthesis and mitochondrial function occur and suggest that these may be responsible for the hepatopathy seen in this model and may thus contribute to the encephalopathy. Eight-day old rat pups were injected subcutaneously with a "flooding" dose of 3,4-$^3$H-valine (VAL) five minutes after intraperitoneal administration of varying doses of MO or saline. Two hours after VAL injection the animals were sacrificed by decapitation, blood was collected, and brains and livers were removed and frozen at -70°C. VAL and its metabolites in the sulfosalicylic acid soluble fraction of the tissues were assayed by LC, HPLC, TLC and LSS. The acid precipitable fractions obtained from liver and brain were washed, delipidated, and assayed for rates of incorporation of VAL into protein. The results show that MO administration caused a biphasic, dose-dependent decrease in accumulation of total VAL metabolites in blood (to 45% of control), brain (45%), and liver (52%), a decrease in protein synthesis in the liver but not the brain (unless the animals were moribund), and an increase in VAL concentration in the liver (123%, paralleling plasma levels) but not the brain. The concentration of nonvolatile metabolites remained unchanged in all groups while the concentration of volatile metabolites (i.e., primarily $^3H_2O$ resulting from mitochondrial oxidation) decreased in a biphasic, dose-dependent manner both in brain and in liver. The depression of VAL metabolism may reflect a mitochondrial insult since volatile metabolite production was primarily affected. The decreased hepatic protein synthesis may reflect an indirect effect resulting from a decrease in mitochondrial energy output. These results contribute to our understanding of the mechanisms of this RLS and indicate that deranged mitochondrial metabolism occurs early in the development of the syndrome and may give rise to both the subcellular and gross pathology of the liver. This may, in turn, contribute to the encephalopathy seen in children suffering from MO toxicity.

This work was supported in part by the Division of Pediatric Intensive Care of Childrens Hospital of Los Angeles, Research Grant NS13515 from NINCDS, and the United Cerebral Palsy Research and Education Foundation.

370.16 $^3$H-GLYCOGENOLYSIS IN THE CEREBRAL CORTEX : CONCENTRATION-DEPENDENT EFFECT OF $K^+$ AND ADENOSINE IN NORMAL MICE AND REDUCED EFFECT OF NOREPINEPHRINE (NE) IN THE TOTTERING MOUSE, A SPONTANEOUSLY EPILEPTIC MUTANT. P.J. Magistretti, P. Hof*, J.-L. Martin* and M. Celio+. Dept. of Pharmacology, University of Geneva, 1211 Geneva, and Anatomisches Institut+, University of Zürich, Switzerland.

$^3$H-glycogen is synthesized in a time-dependent manner from $^3$H-glucose by mouse cerebral cortical slices maintained at 37° in Krebs-Ringer bicarbonate buffer. Neurotransmitters such as VIP and NE promote a concentration-dependent hydrolysis of the newly synthesized $^3$H-glycogen. We have now examined the effects, on $^3$H-glycogen levels, of factors such as $K^+$ and adenosine that are released in the extracellular space during neuronal activity. By increasing extracellular $K^+$ between 5 mM and 53 mM a concentration-dependent $^3$H-glycogenolysis is observed, with an $EC_{50}$ of 10 mM. Significant (15-47 %) glycogenolysis is observed when $K^+$ is increased between 5 mM and 10 mM. These are concentrations of the ion that are reached in the extracellular space upon electrically-induced depolarization. Adenosine promotes a concentration-dependent $^3$H-glycogenolysis with an $EC_{50}$ of 10 µM. Previous investigations have indicated that adenosine concentration in the extracellular space raises, upon electrical stimulation, to 150-400 µM. The rank-order of potency of adenosine agonists indicates that the glycogenolytic effect of adenosine is mediated by receptors of the $A_2$ subtype. These results suggest that $K^+$- and adenosine-mediated glycogenolysis may constitute a mechanism aimed at restoring the energy balance, in rodent cerebral cortex, which would follow an increase in neuronal activity. This mechanism could be contrasted with neurotransmitter-mediated glycogenolysis presumed to constitute an anticipatory mechanism for the local regulation of energy metabolism.

In another set of experiments we have examined the glycogenolytic effect of NE in cerebral cortical slices prepared from the spontaneously epileptic mouse mutant tottering (tg/tg). In this mutant, a selective increase in the number of noradrenergic axons in the terminal fields innervated by the n. locus coeruleus, including the cerebral cortex, has been demonstrated. We have observed that, when compared to wild type (+/+), the concentration-response curve of the glycogenolytic effect of NE is shifted to the right, with a 50-fold increase in the threshold-concentration required to elicit glycogenolysis (+/+ : 10 nm; tg/tg : 500 nM) and in the $EC_{50}$ (+/+ : 100 nM; tg/tg : 5 µM, N=9). Thus a sub-sensitive response of a cellular action of NE can be demonstrated in vitro in the cerebral cortex of tg/tg mice; the metabolic nature of this adaptive change suggests that an impaired capacity of the coeruleo-cortical noradrenergic system in increasing the availability of energy substrates may be related to the expression of the epileptic symptomatology in this mutant.

370.17 MATURATIONAL CHANGES IN ARTERIAL VERSUS BRAIN OXYGENATION IN THE RAT. D.S. Horne, N.R. Krelsman, J.E. Olson, D. Holtzman. Depts. of Pharm., Physiol., Psychiatry/Neurol., Tulane Univ., New Orleans, LA 70112.

Cerebral tissue $Po_2$, cytochrome $aa_3$ ($aa_3$) oxidation, and hemoglobin (Hb) saturation were compared with systemic arterial $Po_2$ and Hb saturation in 10 and 20 day old Sprague-Dawley rats. Animals were anesthetized with 40 mg/kg pentobarbital and either the skull or the femoral artery was exposed. Variations in systemic and cerebral oxygenation were achieved by having the rats respire gas mixtures containing between 0 and 100% $O_2$ (balance $N_2$) via a face mask. Cerebral $Po_2$ was measured polarographically and relative changes in $aa_3$ oxidation or Hb saturation were monitored spectrophotometrically. Tissue $Po_2$, $aa_3$ oxidation, and Hb saturation were expressed as a percentage of full scale signals, defined as the difference between maximum and minimum signals obtained with the various inspired gas mixtures. Arterial blood gas levels were measured in 100 µl samples taken from the abdominal aorta. For both ages, curves relating inspired $O_2$ to cerebral $Po_2$, oxidation of $aa_3$, or Hb saturation were sigmoidal. At inspired $O_2$ concentrations between 5 and 50%, all indices of cerebral oxygenation were greater for 20 day old than for 10 day old rats. For example, at 21% inspired $O_2$, percentages of the full scale signals (mean ± SEM) were:

| AGE | CEREBRAL $Po_2$ | OXIDATION OF $aa_3$ | Hb SATURATION |
|---|---|---|---|
| 10 | 21±9 (n=2) | 47±5 (n=10) | 40±3 (n=6) |
| 20 | 54±2 (n=4) | 73±3 (n= 9) | 68±2 (n=5) |

In contrast, relationships between inspired $O_2$ concentration and systemic arterial $Po_2$ or Hb saturation were the same at both ages. We conclude that age-related differences in cerebral $O_2$ consumption, blood $O_2$ content, or blood flow are responsible for the differences in brain oxygenation. However, cerebral $O_2$ consumption has been shown to increase with development. Therefore, we suggest that either: 1) the ratio of $O_2$ delivery to consumption may change with maturation; or 2) cerebral $O_2$ consumption may be a function of cerebral $O_2$ supply, with the dependence possibly differing with age. Determination of the pertinent mechanisms will be important in the evaluation and treatment of neonatal hypoxia and seizures. (Supported by NS 17443 to NRK, LA Heart Assn. grant to DSH, and NS 20029 and the Psychiatry and Neurology Development Fund to DH and JEO.)

370.18 DIFFERENTIAL DISTRIBUTION OF OXIDATIVE ENZYMES WITHIN SUBNUCLEI OF THE RAT INTERPEDUNCULAR NUCLEUS. G.S. Hamill and B. Fass. Dept. of Anatomy, Hershey Med. Ctr., Penn State University, Hershey, PA 17033, and Dept. of Psychology, Brain Res. Lab., Clark University, Worcester, MA 01610.

Glucose-6-phosphate dehydrogenase (G6-PDH) and succinate dehydrogenase (SDH) are cellular enzymes which catalyze the oxidation of substrates entering the pentose phosphate shunt and citric acid cycle, respectively. These enzymes are differentially distributed throughout the brain within both neurons and neuropil, in amounts which closely correlate with the level of cellular metabolism (Mellgren & Blackstad, 1967).

In this study, histochemical staining for G6-PDH and SDH within individual subnuclei of the interpeduncular nucleus (IPN) was evaluated in adult male rats (300-400 gms). Fresh-frozen cryostat sections (30µ) cut coronally through the IPN were incubated in medium containing polyvinyl pyrrolidone to limit diffusion, and subsequently processed using a standard procedure for demonstrating dehydrogenases (Troyer, 1980). The intensities of G6-PDH and SDH staining within individual IPN subnuclei (Hamill and Lenn, 1983) were quantified by optical densitometry (Nikon Magiscan Image Analysis System).

Optical density (O.D.) scores reveal that G6-PDH and SDH enzymes are differentially distributed within IPN subnuclei, in patterns very different from one another. G6-PDH is most intensely concentrated within the rostral subnucleus, especially within the caudal ovoid regions, followed in order of decreasing intensity by central, intermediate and lateral subnuclei. SDH is most intensely concentrated within the central subnucleus, followed in order of decreasing density by intermediate, rostral, and lateral subnuclei. Control sections incubated without substrate and coenzyme revealed no staining.

The localizations of G6-PDH and SDH in different patterns within individual subnuclei of IPN are nearly identical to the distributions of specific neuropeptide transmitters. The ovoid regions of rostral subnucleus are remarkable for substance P and vasoactive intestinal peptide immunoreactive processes. G6-PDH and SDH staining within the central and intermediate subnuclei indicate possible localizations within horizontal laminations of neuropil, a pattern which precisely overlaps the established distribution of medial habenular axons (Cajal, 1909). Ultrastructural studies are needed in order to determine the precise subcellular localizations of these enzymes within IPN subnuclei.

We thank R.J. O'Connell for use of the densitometer (NS #14453), and Donald Stein for support (FIDIA Contract # CS-185-84).

370.19 AUTORADIOGRAPHIC VISUALIZATION AND CHARACTERIZATION OF [$^3$H]-OUABAIN BINDING TO THE ($Na^+ + K^+$)-ATPase OF RAT BRAIN AND PINEAL GLAND. Mary Lou Caspers*, Rochelle D. Schwartz, Rodrigo Labarca* and Steven M. Paul* (SPON: R.L. Hauger). Clinical Neuroscience Branch, NIMH, Bethesda, MD 20205.

Ouabain binds to the catalytic subunit of ($Na^+ + K^+$)-ATPase and [$^3$H]-ouabain binding can be used as a measure of the number of enzyme molecules present in a given tissue. Previous studies in our laboratory have characterized a "high affinity" population of [$^3$H]-ouabain binding sites in rat and human brain and lesion studies with kainic acid suggest that these binding sites reflect the neuronal form of ($Na^+ + K^+$)-ATPase (Hauger et al., J. Neurochem., in press). In the present study the autoradiographic visualization of [$^3$H]-ouabain binding was determined by incubating 24 µm sections of rat brain in 100 mM Tris buffer (pH 7.4) containing $Na^+$ (10 mM), $Mg^{2+}$ (10 mM), ATP(2 - 10 mM) and [$^3$H]-ouabain (0.02 - 15 µM). In the absence of ATP or in the presence of unlabeled ouabain (50 µM), [$^3$H]-ouabain binding was reduced by 98%. Addition of 30 mM $K^+$ or omission of $Mg^{2+}$ resulted in a 78% and 93% decrease in specific binding, resp. Strophanthidin, digoxin and digoxigenin displaced [$^3$H]-ouabain binding with $IC_{50}$ values of 0.73, 0.48 and 1.4 µM, resp. Scatchard analysis showed a single class of binding sites with an apparent $K_D$ of 339 nM and a Bmax of 34.9 pmol/mg protein. [$^3$H]-Ouabain binding was unevenly distributed throughout the brain with white matter having virtually no specific binding. Using computer assisted densitometry, grain densities in several brain regions were compared to those in white matter. Values reported represent the ratio of grain density of the region to that of white matter. The olfactory nuclei (3.05 ± 0.16), superior colliculus (2.94 ± 0.17), inferior colliculus (2.43 ± 0.10) and dentate gyrus (2.81 ± 0.07) had relatively high densities of [$^3$H]-ouabain binding sites. The pineal gland (2.52 ± 0.12) also had a high density of sites, whereas the caudate/putamen (1.73 ± 0.06) and hypothalamus (2.05 ± 0.24) had lower levels of specific binding. Outer layers of the frontoparietal cortex showed greater specific binding (1.97 ± 0.03) than the inner cortical layers (1.77 ± 0.04). The effect(s) of kainic acid lesions on the autoradiographic visualization of [$^3$H]-ouabain binding in the caudate will be presented. Finally, the binding of [$^3$H]-ouabain in the pineal gland was characterized. Although technically a peripheral tissue, the pineal possessed a high affinity binding site for [$^3$H]-ouabain suggesting a similarity to the CNS (neuronal) binding site.

## STRESS, HORMONES AND AUTONOMIC NERVOUS SYSTEM I

371.1 MECHANISMS OF STRESS-INDUCED GASTRIC ULCERATION IN RAT. Bernt T. Walther and Hans K. Bakke *, Depts. of Biochemistry and Physiological Psychology, Univ. of Bergen, Bergen, N-5000 NORWAY.

Rats maintained in darkness and without food for 23 hours, develop gastric erosions and ulcerations when subjected to forced immobilization. We have found that such stress-induced ulceration depend on a central nervous mechanism since ulcerations are abolished by the central nervous neurotoxin DSP-4 ( N-2-chloroethyl-N-ethyl-2bromobenzylamine).This finding is consistent with numerous reports implicating noradrenalinergic pathways in the central nervous system in stress-caused gastric ulcerations. -In further experiments we have assessed the biochemical changes of the stomach during the ulcerogenic process. No detectable changes could be found in the proteins and glycoproteins of the sub-mucosal layers of the stomach, while the mucosal layer of the stomach corpus exhibited selective depletion in its contents of glycoproteins. In the stomachjuice we found small but significant reductions in the level of pepsin-activity. In trying to reconcile theexperimental findings we have assayed the contents of unsecreted pepsinogens in the gastric walls, after pH-inactivation of activated pepsins present. Stressed animals have 3.2 fold higher contents of pepsinogens in their corpal stomachs than control animals. This situation pertains to stomachs not yet exhibiting massive gastric erosions. Our data suggest that such stomachs are highly susceptible to gastric erosions both because of their diminished protection from mucosal glycoproteins, but also due to the impact of a potential release of an excessive dose of proteolyttic enzymes.However, as DSP-4 treated animals exhibit lowered glycoprotein contents and lowered susceptibility to stress-induced ulceration, the contents of the stomach in terms of pepsinogens and glycoproteins may be necessary but not sufficient for ulceration to occur. Since we have found elevated levels of circulating corticosterone in only stressed control and not in stressed DSP-4 animals, our data suggest that a second central nervous mechanism mediates stress-signals to elicit ulcerations in stress sensitized rat stomachs.

371.2 LATERAL HYPOTHALAMIC LESIONS DIFFERENTIALLY AFFECT THE GASTRIC MUCOSA OF MALE AND FEMALE ALBINO RATS. C.V. Grijalva and B. Roland*. Department of Psychology and Brain Research Institute, UCLA, Los Angeles, CA 90024.

Changes in gastric secretory responses and the production of gastric erosions and ulcers can be induced by various manipulations of certain hypothalamic and limbic system structures and/or their interconnecting pathways. For example, electrolytic lesions of the lateral hypothalamic (LH) area in male rats are immediately followed by vagally-mediated increases in gastric acid secretion and a breakdown of gastric mucosal barrier properties which, in turn, contribute to the formation of stomach ulceration (Grijalva, C.V. et al., Brain Res. Bull. 5, Suppl 1: 19, 1980; Grijalva, C.V., Western J. Med., in press). Invariably, male rats of various strains have been used in previous studies investigating the role of the brain in gastric functions. In the present study the effects of LH lesions in female rats on the stomach were examined.

Three groups of rats were deprived of food overnight. In two groups, one comprised of males and the other of females, animals were anesthetized with sodium pentobarbital (50 mg/kg, ip) and then given bilateral electrolytic LH lesions by passing a 1.2 ma anodal current for 10 sec. Animals were placed in their home cages postoperatively and then sacrificed 24 h later. No food or water was consumed during the 24 h postoperative period. The stomachs were removed, opened along the greater curvature, visually inspected and then analyzed for total area of gastric pathology under a dissection microscope. The third group comprised of female rats served as an unoperated control (2 anesthetized, 3 not) and were similarly food and water deprived.

| Group | N | Total Area of Gastric Pathology ($mm^2$) Glandular | Rumenal |
|---|---|---|---|
| LH Male | 5 | 3.5±0.8* | 0.2±0.2 |
| LH Female | 7 | 0.8±0.5 | 4.7±1.9* |
| C Female | 5 | 0.6±0.3 | 0 |

Male rats with LH lesions primarly displayed hemorrhagic erosions of the glandular mucosa whereas lesioned female rats exhibited edema and crater-like erosions of the rumenal mucosa. Despite the lack of hemorrhagic ulceration to the glandular mucosa in lesioned female rats, the mucosa appeared pathologic and strikingly different from that of the males. The glandular mucosa appeared blanched and sloughing of the superficial lining as well as adherent mucus was notable in 6 of the LH female rats. These results suggest that there may be important dimorphic characteristics involved in the pathophysiology of the gut.
(Supported by UCLA university research grant 3820)

371.3 LACK OF NEUROENDOCRINE RESPONSES TO A MANIPULATION PROCEDURE IN NORMAL VOLUNTEERS. John E. Thornburg, Sheila Barnes*, Myron C. Beal* and Keith T. Demarest. Depts. of Pharmacology and Toxicology, and Family Medicine, Michigan State University, East Lansing, MI 48824.

The objective of this study was to determine if application of a manipulation procedure used commonly in manual medicine causes perturbations in the hypothalamic-pituitary-adrenal axis or sympathetic nervous system as reflected by plasma levels of norepinephrine, cortisol, glucose and β-endorphin. For comparison, the effects of a 1 minute cold pressor test and orthostasis on these neuroendocrine parameters were also determined.

Ten healthy volunteers ranging in age from 20-27 years and who gave informed consent were tested as follows: A heparin lock was established in an antecubital vein. Following 45 minutes in a supine position, baseline blood samples were obtained. A manipulation procedure consisting primarily of stretching of the left and right thoracic paravertebral muscles was applied by one of the investigators (MCB) for 4 minutes. Blood samples were obtained after 1, 5 and 30 minutes. Subjects then stood erect for 10 minutes to determine the effects of orthostasis and blood samples were obtained at 5 and 10 minutes. Subjects were then in a sitting position for 30 minutes before placing a hand (opposite to the i.v.) in ice water (0°-4°C) for 1 minute. Blood samples were obtained at 0, 1 and 5 or 10 minutes. Plasma norepinephrine were determined using a catechol-o-methyltransferase radioenzymatic procedure. Plasma cortisol and β-endorphin were measured using radioimmunoassay kits from Immuno-Nuclear Corp. (Stillwater, MN).

The manipulation procedure did not alter any of the neuroendocrine parameters measured using within subjects comparisons. In contrast, the cold pressor test caused significant increases in plasma levels of cortisol and norepinephrine. Orthostasis increased only plasma levels of norepinephrine.

In conclusion, application of a manipulation procedure to young pain-free individuals did not alter indices of pituitary-adrenal or sympathetic nervous system activity. (Supported by a Biomedical Research Grant from the College of Osteopathic Medicine, Michigan State University.)

371.4 NEUROENDOCRINE RESPONSE TO MAXIMAL TREADMILL EXERCISE. M.A. Oleshansky, R.H. Herman*, J. Zoltick*, P. Kluge*, E.H. Mougey* and J.L. Meyerhoff. Dept. of Medical Neurosciences, Div. of NP, Walter Reed Army Institute of Research and Cardiology Div., Dept. of Medicine, Walter Reed Army Medical Center, Washington, D.C. 20307-5100.

Plasma norepinephrine (NE), epinephrine (EPI), β-lipotropin (β-LPH), β-endorphin (β-END), adrenocorticotropin (ACTH), cortisol and cAMP levels were measured in healthy volunteers before, during and after maximal treadmill exercise to characterize the extent and duration of neuroendocrine and neurochemical responses to physical stress. Since previous studies have noted an effect of prior physical training on β-END responses, a range of highly fit to non-fit subjects was studied.

A maximal exercise treadmill test (ETT) was performed on twenty healthy male subjects, age 41±2.2 years (mean±S.E.M., range 20-61 years). The exercise was performed until exhaustion on a treadmill at a constant rate of 3.3 mile/hr with a 5% increase in grade every three min. Maximal ETT time provided an index of fitness. Plasma was collected: (A) thirty minutes after resting supine; (B) fifteen min after standing, just prior to exercise; (C) upon exercising to a heart rate of 140 beats/min; (D) immediately following maximal exercise; and (E) fifteen min after exercise. NE and E were extracted from 1 ml of plasma and assayed by HPLC. β-LPH and β-END were extracted from 1 ml of plasma and assayed by RIA. ACTH, cortisol and cyclic AMP were assayed by RIA.

Mean ETT time to heart rate 140 was 9.0±0.6 (range 3.0-13.0) and mean maximal ETT time was 15.0±0.6 min (range 8.9-19 min). Values for plasma catecholamines, proopiomelanocortin-derived peptides, cortisol and cyclic AMP (mean ± S.E.M.) at the various time points are given below.

| | A | B | C | D | E |
|---|---|---|---|---|---|
| NE(pg/ml) | 279±32 | 567±54 | 1270±129 | 1929±194 | 600±60 |
| EPI(pg/ml) | 85±9 | 120±15 | 239±32 | 285±42 | 136±20 |
| β-LPH(pg/ml) | 56±4 | 60±5 | 74±5 | 219±26 | 198±30 |
| β-END(pg/ml) | 3.4±0.4 | 4.3±0.6 | 6.6±0.9 | 33.0±5.0 | 26.1±5.1 |
| ACTH(pg/ml) | 22±8 | 28±9 | 36±9 | 68±10 | 68±15 |
| CORTISOL(ug%) | 6.4±0.7 | 6.3±0.5 | 7.5±0.7 | 6.6±0.8 | 10.0±0.9 |
| cAMP(pg/ml) | 28±2 | 31±3 | 42±5 | 47±5 | 37±3 |

Plasma values were poorly correlated with exercise time to heart rate 140 or maximal ETT time with the exception of NE at time C which correlated strongly with exercise time to heart rate 140; R value of 0.60 ($p<0.02$). The mean changes of plasma β-LPH and β-END and ACTH levels from maximal exercise (D) to recovery (E) were -20.7±22.6 (range -136 to +243), -7.0±4.6 (range -33 to +37) and -7.5±7.6 (range -42 to 70) pg/ml respectively. The change of plasma β-LPH, β-END and ACTH levels from maximal exercise to recovery were inversely correlated with maximal ETT time with R values of -0.48 ($p<0.05$), -0.44 ($p<0.07$) and -0.52 ($p<0.03$).

We find that changes in the plasma levels of the major proopiomelanocortin-derived peptides during a brief recovery period after exercise are inversely correlated with fitness as expressed by maximal ETT time.

371.5 NEUROENDOCRINE RESPONSE TO STRESS IN SYRIAN GOLDEN HAMSTERS. B.N. BUNNELL, K.M. LEVY*, S.M. GILCHRIST*, A.A. GRANBERRY*, E.H. MOUGEY*, W.L. GAMBLE* AND J.L. MEYERHOFF. Dept. Psychology, U. Georgia, Athens, GA 30602 and Dept. Med. Neurosciences, Walter Reed Army Institute of Research, Washington, DC 20307-5100.

Despite the widespread use of Syrian golden hamsters as subjects for research on a large number of neuroscience problems, little is known about the neuroendocrine response to stress in this species. The data that are available indicate that cortisol is the primary glucocorticoid secreted by hamsters, that hamsters have the lowest adrenal corticoid secretion rate of any laboratory animal studied, and that cold stress produces significant increases in corticoid secretion (Schindler, W.J. and Knigge, K.K., Endocrinology, 65:739, 1959; Frenkel, J.K., et al., Lab. Invest., 14:142, 1965).

The present study investigated changes in plasma cortisol, ACTH, and β-endorphin levels in male and female hamsters in response to stress induced via footshock. Sixteen male and 16 female adult golden hamsters were handled daily for 14 days prior to the experiment. In the stress condition, 8 males and 8 females were subjected for 20 min to intermittent 5 sec, 1 mA footshock delivered on a 60 sec variable time schedule in sound attenuated shock chambers. The remaining animals served as home cage controls. Animals were sacrificed by decapitation and trunk blood was collected in heparinized tubes. After centrifugation, plasma was stored at -18°C in tubes containing 50 µg trasylol until radioimmunoassays for plasma hormones were carried out. The experiment was conducted over two days, with 8 males and 8 females used each day. The schedule was arranged such that animals were sacrificed between 9:00 and 11:00 AM; they were housed under a 12:12 LD cycle with lights on at 6:00 AM.

Plasma corticosterone levels were extremely low, and did not increase in response to footshock. Plasma cortisol levels were low in male controls, but were significantly elevated in response to the stressor. Cortisol responses in females paralleled those of males on day 1. On day 2, however, females in the control group had high levels of cortisol. This may have been an effect of endogenous estrogen.

| | PLASMA CORTISOL (µg/100ml) | | | |
|---|---|---|---|---|
| | Male | | Female | |
| | Day 1 | Day 2 | Day 1 | Day 2 |
| Shock | 4.78 ± 1.36 | 4.76 ± 0.36 | 3.74 ± 0.42 | 2.29 ± 0.46 |
| Control | 0.58 ± 0.21 | 0.21 ± 0.27 | 0.21 ± 0.08 | 3.88 ± 1.09 |

There were significant positive correlations between cortisol levels and ACTH (+.72) and β-endorphin (+.71). ACTH and β-endorphin levels had a correlation of +.77.

371.6 EFFECT OF DISHABITUATION AFTER INTERRUPTION OF REGULARLY REPEATED COLD EXPOSURE IN C57BL/6J MICE. M.I.Talan and B.T.Engel*. Lab. of Behavioral Sciences, Gerontology Research Center, National Institute on Aging, NIH, Baltimore, MD 21224.

We defined cold tolerance as the rate of decline of colonic temperature per min during a 3-hr period of restraint in an ambient temperature of 10°C (Talan, M.I. and Engel, B.T., Exp. Gerontol., 19:79, 1984). In Experiment 1, two groups of male C57BL/6J mice, 10 animals each, aged 12 mo at the beginning of the study were exposed to repeated cold stress tests with 2-wk intervals over 4-6 mo (Talan, M.I., Engel, B.T. and Whitaker, J.R., J. Gerontol., 40:8, 1985). In group 1 tests number 4 and 8, were omitted so there was a 4-wk interval preceding tests 5 and 9, whereas group 2 was always tested at 2-wk intervals. Cold tolerance was not different between groups for any test, $F_{(1,17)}=1.24$, $p=0.28$, except 5 and 9, where cold tolerance was significantly worse for group 1 ($p's<0.05$). This effect was limited to cold tolerance and did not include baseline colonic temperature. Furthermore, cold tolerance returned to previous levels in subsequent tests following restoration of the 2-wk testing cycle. In Experiment 2, mice, which participated in Experiment 1, underwent stereotaxic implantation of concentric electrodes in "rewarding" areas of the lateral hypothalamus. Previously, we had shown that electrical stimulation in these areas could retard age-related deterioration of cold tolerance in 30-mo old C57BL/6J mice (Talan, M.I., Engel, B.T. and Whitaker, J.R., Physiol. Behav., 33:969, 1984). Seven animals in group 1 and seven in group 2 were presented the schedule of cold exposure as in Experiment 1; test 5 was skipped in group 1 and test 8 was skipped in both groups. During 4-wk interval between tests 4 and 6, group 1 received 30 min daily sessions of brain stimulation. Both groups demonstrated a decline of cold tolerance on test 9; however, test 6 was not different between groups. Therefore, electrical stimulation of a "rewarding" area of the hypothalamus seems to supress the effect of dishabituation following interruption of regular cold exposures.

371.7 ADRENAL CATECHOLAMINE SECRETION ASSOCIATED WITH NATURAL AGGRESSIVE BEHAVIOR IN THE CAT. S.L. Stoddard-Apter, V. Bergdall* and B.E. Levin. Dept. of Anatomy, Indiana Univ. Sch. of Med., Ft. Wayne, IN 46805 and Dept. of Neurosci., N.J. Med. Sch., Newark, NJ 07103.

This study was designed to evaluate the contribution of the adrenal medulla to the sympatho-adrenal (SA) response during naturally elicited aggressive behavior in the cat. An arterial cannula was placed in the aortic arch through the left internal carotid for the continuous monitoring of heart rate and blood pressure. Two cannulae were placed in the inferior vena cava (IVC) through the left external jugular vein for the withdrawal of venous blood samples. Within the IVC, the tips of the two cannulae were placed 8-10 cm apart, with the proximal line positioned to collect blood from the effluent of the left adrenal vein. The sympatho-adrenal response was determined following both offensive and defensive forms of aggressive behavior. Offense (pure attack) was observed in trained, adult male cats, while defense was evoked by exposure to either a large, barking dog or a hypothalamically stimulated cat. Blood samples were withdrawn simultaneously from both venous cannulae during two baseline periods prior to aggressive behavior, and 0.25, 0.5, 0.75, 1 and 3 min following the behavior. Levels of plasma norepinephrine (NE) and epinephrine (E) were determined by radioenzymatic assay. The difference in the levels of catecholamines (CAs) in plasma samples collected proximal and distal to the output of the left adrenal vein served as an index of the activity of the adrenal medulla. Initial studies of defense behavior found that both NE and E increased following the behavior. Two observations implicated the adrenal medulla as the source of both CAs: the peak increases in these CAs were concomitant, and they occurred first in the plasma sample collected from the proximal cannula. Furthermore, plasma CA levels reached a peak immediately following the behavior. The subsequent decrease in CA levels in the plasma was similar to a half-life curve, suggesting that activation of the adrenal medulla was restricted to the period of the behavioral stimulus. These preliminary data support a previous observation that, in the cat, hypothalamically mediated aggressive behavior may involve adrenal medullary release of levels of NE equal to, or greater than, the levels of E.

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371.8 DIETARY CALCIUM, BEHAVIORAL STRESS, AND HYPERTENSION IN THE SPONTANEOUSLY HYPERTENSIVE RAT. M.S. Muntzel*, P.E. Huie,* D.C. Hatton*, J.A. Metz*, & D.A. McCarron* (SPON: D. Trune), Depts of Medical Psychology & Nephrology, Oregon Health Sciences University, Portland, OR 97201.

The interaction of dietary calcium with both chronic and acute stress was studied in two experiments. In the first, 50 6-week-old male spontaneously hypertensive rats (SHRs) were randomly assigned to one of 3 diet conditions: Normal (1.0% Ca, .25% Na); High (2.0% Ca, 1.0% Na); Low (.10% Ca, .25% Na); and to either a Social Stress (SS) or Control (C) condition, thus making a 2 x 3 factorial design. Chronic stress was produced by housing the 8 male subjects with 8 female SHRs in a cage consisting of a 1m square central arena with two smaller cages on each side. Control subjects were housed in groups of 3 and 4 in standard lab cages. Tail-cuff systolic BP measurements were taken biweekly for 13 weeks. Over the last 4 measurements mean BP (mmHg) was significantly higher ($p < .01$) in L diet rats (SS=215, C=200) than in N (SS=190, C=183) or H diet rats (SS=184, C=183) in both SS and C groups, while N and H rats did not differ from each other at either stress level. BP change scores computed relative to the week 1 baseline showed a significantly larger stress effect ($p < .01$) in L diet rats relative to that effect in N and H diet rats. Moreover, between-groups t-tests revealed that S rats had lower serum ionized $Ca^{+2}$ (mMol/l: S=1.10; C=1.18; $p < .01$) than controls.

These results suggested a synergistic interaction between dietary Ca and chronic stress in augmenting the development of elevated pressure in the SHR. Since it is suspected that the development of hypertension in SHRs is partly due to their exaggerated SNS responses to environmental stimuli, such as would be afforded by the frequent conspecific encounters in the social stress situation, it was hypothesized that the observed interaction between diet and chronic stress might in fact reflect an effect on acute CV responsivity to environmental stressors. To test this hypothesis, a second experiment was performed in which 30 6-week-old male SHRs were randomly assigned to the N, H, or L diets as above. After 11 weeks on the diet all rats were classically conditioned with 24 trials of a 20 sec tone followed by a 500 msec electric shock. Mean arterial pressure (MAP) was measured by femoral catheter during baseline (10 sec pre CS) and during successive 2 sec periods of the CS. Change scores were computed for each counting period by subtracting MAP for that period from the baseline score. While all groups showed pressor responses to the CS, t-tests showed the diet groups to be different from one another ($p < .05$) at all counting periods except the first, with the Ca deprived, L diet rats having the greatest responses and the H diet rats the smallest.

371.9 PSYCHOSOCIAL STRESS ALTERS MAMMARY TUMOR GROWTH IN MICE. J. Weinberg, A. Scarth* and J.T. Emerman*. Dept. of Anatomy, Faculty of Medicine, University of British Columbia, Vancouver, B.C. V6T 1W5.

The influence of psychosocial stress on mammary tumor incidence and growth was studied. Tumor cells were obtained from the transplantable androgen-responsive Shionogi mouse mammary carcinoma. Male mice (DD-S) were injected (s.c.) in the interscapular region with 4 x $10^6$ cells in 0.2 ml medium. Four groups (n = 10-12 per group) were formed immediately following injection: 1) Isolate to Group (IG) - males raised singly housed from 30 days of age were moved to non-sib groups of 4-5 males; 2) Isolate to Male-Female Pair (IP) - males raised singly housed were caged with a female; 3) Group to Isolate (GI) - males raised in sibling groups of 2-3 were separated and housed singly; 4) Group to Group (GG) - males raised in sibling groups of 2-3 remained in their groups. In addition, half the animals in each of the 4 housing groups were subjected daily to the acute stress of placement into a novel environment (15 min/day, 5 days/week). Mice were palpated twice weekly and time until appearance of palpable tumors was recorded. Once palpable, tumors were measured using calipers and tumor weight calculated. Observations were continued for 23-24 days following injection of cells. On the day of termination, animals were decapitated and trunk blood collected for determination of plasma levels of corticosterone. Tumors and spleens were then removed and frozen.

Tumor growth was significantly altered both by housing condition and by the acute stress of daily exposure to novel environments. Tumor growth was most rapid in GI animals and least rapid in IG animals. By the day of termination animals in each of the 4 housing conditions differed significantly from each other in mean tumor weights (GI>GG>IP>IG, ps <.05).
In addition, animals subjected to daily novelty stress developed larger tumors than animals in the unstressed group ($p < .05$), with the greatest effect appearing in singly housed animals. There was no chronic change in basal levels of corticosterone due to housing condition. However, animals subjected to novelty stress showed significant corticoid elevations, even following the 16th exposure.

Thus both housing condition and novelty stress significantly affected rate of tumor growth. Pituitary-adrenal activation may have some role in mediating the effects of exposure to novel environments. The immunocompetence of these animals is currently being investigated. In addition, we are examining the role of androgens in differential tumor growth.

371.10 **MODIFICATION OF PITUITARY-ADRENOCORTICAL RESPONDING TO STRESS BY CHOLINERGIC BLOCKADE.** **M. Alharbi* and R.P. Maickel** (SPON: G.K.W. Yim). Dept. of Pharmacology and Toxicology, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, IN 47907.

**A previous report from this laboratory (Odio and Maickel, Fed. Proc. 42: 726, 1983) had shown that pretreatment of rats with a cholinergic blocking agent (atropine) potentiated the response of the pituitary-adrenocortical system to acute exposure to stressful stimuli such as immobilization, cold exposure, or inescapable random foot shock. This increased response was manifested by increases in magnitude and duration of elevated levels of plasma corticosterone (PCS) during 1 to 4 hours of exposure to the stressful stimuli. The present paper describes studies of the effects of cholinergic blockade on the responses of PCS, plasma fatty acids (PFA) and plasma glucose (PGL) in rats exposed to repetitive periods of stressful stimuli on an irregular/unpredictable schedule for 16 days. Two stressful stimuli were used: isolation in a novel environment (ISOL) and immobilization (IMMB). In addition, two control groups were used: no drug (NODR) and placebo injected (INJC). The first studies used atropine (5.0 mg/kg, i.p.) as the cholinergic blocker; it was administered 15 minutes prior to the start of a stress session. Blood samples were obtained by orbital sinus puncture immediately before and at the termination of the stress session. At least 72 hours separated repeated tests in the same animal. Atropine pretreatment significantly increased the response of PCS to IMMB and INJC, but did not produce a significant change in the response to ISOL. These results suggest that there may be a differential role of cholinergic systems related to either type or severity of stress. With regard to the PFA response, atropine had no significant effects on animals exposed to either ISOL or IMMB. In contrast, atropine pretreatment significantly increased the PGL response to INJC and ISOL, but had no significant actions on the response to IMMB. Thus, cholinergic blockade appears to have stress-specific and response-specific actions; whether this involves effects on specific neuronal pathways remains the subject of future study. (Supported in part by the Saudi Arabian Educational Mission and by American Cancer Society CH194A).**

371.11 ATROPINE SULFATE AND 2-PYRIDINE ALDOXIME METHYLCHLORIDE ELICIT STRESS-INDUCED CONVULSIONS AND LETHALITY IN MICE. B.A. Donzanti, M.D. Green*, E.I. Shores* and C.K. Burdick*. U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010-5425.

Atropine sulfate and 2-pyridine aldoxime methylchloride (2-PAM) are currently the treatments of choice for organophosphate chemical warfare agent intoxication. These drugs appear to be relatively nontoxic even after repeated administration of high doses. However, most toxicological studies have been performed under nonstressful environmental conditions which may not always be applicable to the soldier in the field. It is more likely that the use of these compounds will occur under stressful situations. Since stress has been shown to modify the pharmacological actions of some drugs, it was the intent of this study to investigate changes in the behavioral toxicity of atropine and 2-PAM induced by physical stress.

Male ICR Swiss mice were injected intramuscularly (i.m.) with either vehicle or drug and placed individually in beakers filled with cold water (16°C) from which there was no escape. Control animals receiving sterile water showed no adverse behavioral signs other than fatigue. Similarly, the administration of low doses of atropine (0.1, 5.6 and 11.2 mg/kg) or 2-PAM (5, 12.5 and 25 mg/kg) prior to the stress did not produce any adverse behavioral effects. In contrast, higher doses of atropine (80.6 and 150 mg/kg) or 2-PAM (50 and 100 mg/kg) precipitated violent tonic-clonic convulsions and death within 15-20 minutes while being stressed. Moreover, dose combinations of atropine and 2-PAM, as low as 0.1 and 12.5 mg/kg, respectively, elicited convulsions. Similar results were observed when N-methyl atropine was substituted for atropine. Pretreatment with an alpha-adrenergic blocking agent, phenoxybenzamine (6 mg/kg, i.p.), completely blocked stress-induced convulsions and death elicited by dose combinations of atropine (11.2 mg/kg) and 2-PAM (25 mg/kg); however, a beta-adrenergic blocking agent, propranolol (20 mg/kg, i.p.), was totally ineffective. The cholinomimetic, pilocarpine (5 mg/kg, i.m.), did not alter the incidence of atropine and 2-PAM stress-induced convulsions but did provide significant protection ($p < 0.05$) against the ensuing lethality. Diazepam (1.25 mg/kg, i.m.) showed significant protection ($p < 0.05$) against both the precipitation of convulsions and lethality. Changes in plasma glucose levels and rectal body temperature did not appear to account for the precipitation of convulsions.

The above results suggest that alpha-noradrenergic mechanisms are involved in the precipitation of atropine and 2-PAM stress-induced convulsions and that cholinergic mechanisms may be involved in the lethality following convulsions.

371.12 BLOOD GLUCOSE REGULATION DURING STRESS IN STREPTOZOTOCIN TREATED MICE. L.J. Leedom*, W.P. Meehan*, T. Nagayama*, and A. Zeidler* (SPON: R. Binggeli). USC Sch. of Med., Los Angeles, Calif. 90033.

Previously we have shown that the resident-intruder paradigm may be used to demonstrate altered social behavior in diabetic mice. In this study we used this experimental design to investigate the effects of psychosocial stress on blood glucose regulation in male diabetic mice.

69 adult male Swiss Webster mice were used in this study. Diabetes mellitus was induced with the diabetogenic drug streptozotocin (SZ). To obtain varying degrees of diabetes, several doses of I.P. SZ were given: single dose 140mg/kg (n=18) and 200mg/kg (n=18) and multiple dose 200 mg/kg over 5 days (40mg/kg/day) (n=9). 25 mice received vehicle and served as controls. SZ treated mice were used 14-28 days after injection. Experiments were done on fed animals between 10 A.M. and 2 P.M. Mice were bled by retro-orbital puncture to obtain baseline glucose values, and introduced singly into the home cages of individually housed, aggression trained, resident males. The behaviors of the mice were videotaped while they were allowed to interact for 10 minutes. After the 10 min. stress period the test animal was removed and immediately bled to obtain a post stress blood sample. To control for bleeding effects, mice (n=55) were also bled, returned to their home cages for 10 minutes, and bled again. Glucose was analyzed using a Beckman Glucose Analyzer.

Fed baseline blood glucose of the control mice was 177±24 mg/dl s.d. In these mice bleeding alone without psychosocial stress increased blood glucose from 177±25 to 197±41 mg/dl with an average increase of 20±40 mg/dl ($p<0.05$ paired t test). In controls post-stress mean blood glucose was increased from pre-stress (179±21 to 246±41 mg/dl). The average stress glucose increase was significant (66±44 mg/dl $p<0.0005$).

In SZ treated mice with baseline glucose 120-250 mg/dl (n=11) bleeding alone produced an increase in blood glucose from 202±25 to 243±51 mg/dl, the mean increase of 47±37 mg/dl was significant ($p<0.0005$). Following psychosocial stress an even greater increase in blood glucose 218±55 (pre-stress) to 320±71 mg/dl (post stress) was seen. The mean rise was 102±42 mg/dl ($p<0.0005$). Mice which were both bled and stressed (n=8) were examined to see how well bleeding trial glucose changes correlated to stress trial glucose changes. There was a 0.97 correlation between increases from bleeding and increases from stress ($p<0.001$). In SZ treated mice with baseline blood glucose 251 to 699 mg/dl (n=19), bleeding produced an increase from 524±94 to 567±107 mg/dl, with an average increase of 44±38 mg/dl ($p<0.0005$). Stress increased blood glucose from 487±123 to 585±139 mg/dl, with an average increase of 98±61 mg/dl ($p<0.0005$). In mice which were both bled and stressed there was a positive correlation between increases in glucose from bleeding alone and increases due to stress ($r=0.35$). In SZ treated mice with baseline blood glucose >700 (n=14), neither bleeding alone nor stress increased blood glucose significantly: bleeding alone: 783±36 to 771±78 mg/dl, and stress: 763±37 to 782±49 mg/dl.

In summary, 1) In control mice blood glucose increased significantly to psychosocial stress and to bleeding alone. 2) Mildly or moderately diabetic mice sustained a significant rise in blood glucose from bleeding alone, and a 2x greater increase from psychosocial stress. 3) In severely diabetic mice, significant increases in blood glucose did not occur with bleeding alone or psychosocial stress.

In conclusion, the degree of change in blood glucose during stress in mice was shown to be related to both 1) the severity of the stress and 2) the baseline diabetic state.

371.13 CORTICOSTERONE, NOVELTY-INDUCED HYPERGLYCEMIA AND AND CHLORDIAZEPOXIDE. C.F. Flaherty, G.A. Rowan*, and L. Pohorecky.* Psychology Dept. and Center for Alcohol Studies, Rutgers University, New Brunswick, N.J. 08903.

Previous studies have shown contradictory results when exogenous insulin is administered as a US in studies of glycemic conditioning. Some have found a hypoglycemic CR on test trials for conditioning (e.g. Woods, S., Am.J. Physiology, 223:1424, 1972), whereas others have obtained a hyperglycemic CR (Siegel, S., J.Comp. Physiol. Psych., 89:189, 1975). The hyperglycemic CR has been interpreted as a conditioned compensatory response, a learned response which functions to oppose the effects of the US.

We have previously obtained both hyperglycemic and hypoglycemic CR patterns when different environmental contexts are used as the CS. Our interpretation that the hyperglycemic CR represented a conditioned stress response was supported by the finding that chlordiazepoxide (CDP) administration as a pretreatment resulted in a hypoglycemic CR in the environment previously shown to elicit hyperglycemic CRs (Flaherty, et.al., Physiol. & Behav., 33:595, 1984).

In the present experiment we show that different degrees of environmental novelty, relative to the animals' housing conditions, lead to graduated increases in corticosterone secretion and plasma glucose levels. The largest increase in both occurred when the rats were moved to the context in which a hyperglycemic CR was obtained in prior experiments. The context in which hypoglycemic CRs were previously obtained led to a significantly smaller rise in plasma glucose and corticosterone. CDP (5 and 10 mg/kg) decreased the degree of hyperglycemia elicited by environmental novelty. The results are taken to support the hypothesis that the conditioned hyperglycemic CR is a conditioned stress response related, in part, to degree of environmental novelty.

371.14 ADRENAL DEMEDULATION ATTENUATES AND CORTICOSTERONE SYNTHESIS INHIBITION ENHANCES THE GLYCEMIC RESPONSE TO BRIEF FOOTSHOCK STRESS. R.J. Bialik and D.C.S. Roberts. Dept. of Psychology, Carleton Univ., Ottawa, Canada, K1S 5B6.

Many labs have reported that manipulation of adrenal hormones can impair performance in learning situations involving stress. It is possible that these deficits are due to an alteration in hormonally mediated blood glucose responses. Studies on isolated hepatic tissue have suggested that both epinephrine (E) and corticosterone (CORT) promote glycogenolysis in the liver which would lead to an increase in blood glucose levels. The present study investigated the roles of these two hormones in the blood glucose response to a stressfull situation similar to that found in some learning tasks.

Blood glucose was measured in a small sample of blood taken from the tail before and at various intervals after a series of footshocks (15 X 0.5 mA shocks over a 5 min period). Sham operated rats displayed a 40% increase in blood glucose levels 10-20 min following footshock. In contrast, adrenalectomized rats failed to show an increase in blood glucose levels. This blockade was also observed in the adrenal demedulated rats, although they showing a CORT response similar to control animals (300% increase following stress). These data indicate that, in the paradigm used here, E is necessary for the stress-induced blood glucose response.

In a second experiment, rats were treated with an inhibitor of CORT synthesis (Metopirone, 50 mg/kg ip) 2 hours before subjecting them to footshock stress. We found that this drug treatment enhanced the blood glucose response to footshock stress compared to vehicle treated rats (75% vs 40% increase). These data indicate that CORT may function to attenuate the blood glucose surge in response to a mild stressor. This suggestion is consistent with recent views that CORT may function in a general manner to dampen adaptive responses to stress.

We conclude that the increase in blood glucose caused by mild footshock stress is mediated by release of E from the adrenal medulla. Furthermore, in our paradigm, CORT functions to dampen this hyperglycemic response to footshock stress. Since the number and intensity of footshocks in this experiment are similar to those used in behavioral studies, these hormonal alterations in the blood glucose response to mild stress may explain deficits in aversive conditioning caused by manipulation of these hormones (eg. Bialik, Pappas & Roberts, Behavioral Neuroscience, 98 (1984) 847). (Supported by the Medical Research Council and an Ontario Mental Health Foundation Studentship to RJB).

372.1 STRESS-LIKE NEUROCHEMICAL AND BEHAVIORAL EFFECTS OF CORTICOTROPIN-RELEASING FACTOR (CRF) A.J. Dunn and C.W. Berridge*. Dept of Neuroscience, Univ. of Florida Coll. of Med., Gainesville, FL 32610.

A primary function of CRF is to mediate the physiological release of ACTH from the anterior pituitary. However, CRF-containing neurons in the hypothalamus project to other brain regions, and intracerebrally injected CRF has distinct behavioral effects, so that CRF may act directly on brain cells. We have studied the behavioral and neurochemical responses of mice to both central (lateral ventricle) and peripheral (SC) administration of CRF. ICV injection of 1 µg (but not 0.2 µg) CRF induced abrupt brief bouts of hyperactivaty, resembling that reported in rats (Sutton et al., Nature 297:331, 1982). By contrast, SC CRF (1 or 10 µg) caused hypoactivity and periods of inactivity.

The behavioral response to ICV CRF resembles that which occurs in novel situations, so we tested whether CRF produces a response of cerebral catecholamine systems like that of stress. Using HPLC with electrochemical detection we measured the cerebral content of dopamine (DA) and norepinephrine (NE), and their respective major catabolites, dihydroxyphenylacetic acid (DOPAC), and 3-methoxy,4-hydroxyphenylethyleneglycol (MHPG), 30 minutes after CRF. ICV CRF (0.2 or 1 µg) significantly increased DOPAC and DOPAC/DA ratios in prefrontal cortex, caudate-putamen, septum, hypothalamus, and brain stem relative to animals injected with saline. MHPG and MHPG/NE ratios were also increased in the hypothalamus, hippocampus, and brain stem. By contrast, SC CRF (1 or 10 µg) markedly increased MHPG and MHPG/NE ratios in all brain regions, but DOPAC/ DA only in the frontal cortex. These results bear a close resemblance to those observed following stressors such as footshock. At low shock intensities, DOPAC/DA is selectively elevated in frontal cortex, hypothalamus, and brain stem; whereas higher intensities or restraint treatment causes widespread increases in DOPAC/DA. MHPG and MHPG/NE is increased in most brain regions by any stressor.

Plasma corticosterone was not significantly altered by ICV CRF relative to saline, but was markedly elevated by SC CRF.

These results suggest that CRF administered by either route activates CNS DA and NE systems, and that the responses resemble those in stress. However, the effects of ICV CRF on noradrenergic systems are less marked (relative to saline) than those of stress. It is possible that intracerebral CRF mediates the CNS activation of catecholamines, and that these changes underlie the behavioral responses. However, ICV CRF cannot be considered a stressor because it fails to activate the hypothalamic-pituitary-adrenal (HPA) axis. In fact, it fractionates the normal stress response. Peripherally administered CRF may trigger a global stress response because it stimulates the CNS DA and NE systems and the HPA axis.

(Supported by USPHS grant MH25486.)

372.2 STRESS-INDUCED ALTERATIONS IN CORTICOTROPIN RELEASING FACTOR-LIKE IMMUNOREACTIVITY (CRF-LI) IN RAT BRAIN. M. A. Smith, P. B. Chappell*, G. Bissette, J. Ritchie, C. D. Kilts, and C. B. Nemeroff. Department of Psychiatry, Duke University Medical Center, Durham, N.C. 27710.

Corticotropin releasing factor (CRF) is believed to play a crucial role in regulating endocrine, autonomic and behavioral responses to stress. Recent evidence indicates that CRF-like immunoreactivity is widely distributed throughout the CNS. In the current study, we investigated the effect of acute and chronic stress on CRF-LI concentrations in 36 different rat brain regions.

A control group of 10 male rats was handled daily for 2 weeks. The acute stress group was subjected to 3 hrs of immobilization stress at 4°C; the chronic stress group was exposed to a series of stressors (overcrowding, cold, forced swimming etc.) for 2 weeks prior to decapitation. Brains were frozen, 300 um coronal sections were cut on a cryostat, and 36 brain regions were microdissected by the technique of Palkovits. The brain samples were sonicated in 1 N HCL, centrifuged, and the supernatants lyophilized and assayed for CRF-LI by radioimmunoassay, using a specific antiserum to ovine CRF.

Measurable CRF-LI (pg/mg protein ± SEM) was found in most of the 36 regions. Acute stress reduced CRF-LI in the median eminence/arcuate nucleus from 5050 ± 670 to 2420 ± 530 ($p < .01$) and in the medial preoptic nucleus from 143 ± 11 to 97 ± 5 ($p < .01$). In contrast, acute stress produced a significant increase in CRF-LI in the locus ceruleus from 147 ± 9 to 306 ± 50 ($p < .001$). In chronically stressed animals, CRF-LI was elevated not only in the locus ceruleus but also in the anterior hypothalamus and periventricular nucleus. Reduced concentrations were found in the dorsal vagal nucleus from 194 ± 15 to 128 ± 11 ($p < .025$). The median eminence of chronically stressed rats had similar reductions of CRF-LI as in the acutely stressed animals. Other areas which contained relatively high concentrations of CRF, such as the amygdala and raphe nuclei, were unaffected by either acute or chronic stress. Plasma corticosterone and ACTH were significantly elevated during acute stress while only corticosterone and adrenal weights were elevated following chronic stress.

These results suggest that stress may alter concentrations of CRF-LI in various brain nuclei. In addition to the hypothalamus, stress-induced changes in CRF-LI were observed in the locus ceruleus and the dorsal vagal nucleus, and these areas may play a role in mediating the central nervous system response to stress. (Supported by NIMH MH-39415)

372.3 PLASMA NOREPINEPHRINE, EPINEPHRINE AND CORTICOSTERONE STRESS RESPONSES TO RESTRAINT IN INDIVIDUAL MALE AND FEMALE RATS, AND THEIR CORRELATIONS. G.T. Livezey, J.M. Miller and W.H. Vogel, Dept. Pharmacology, Thomas Jefferson Univ., Phila. PA 19107

Many studies have shown that the plasma levels of norepinephrine (NE), epinephrine (E), and corticosterone (C) rise markedly during exposure to stress. Most frequently, NE and E, and C levels were measured in seperate studies and reported only as group means of unstressed and stressed animals. However, more recently it has become evident that rats from the same strain and reared almost identically show marked, but individually constant, differences in the NE, E, and C responses to stress (Pharmacol.Biochem.Behav. 13:129-131, 1980). We examined the stress response of NE, E and C in the same individuals and compared these values within and among themselves, as well as correlations within and among E, NE, and C. Male and female Sprague Dawley rats were fitted with chronic jugular catheters and individually housed with ad lib food and water. After 24 hours recovery, blood samples (0.3ml) were taken before (time 0) and during 30 minutes of restraint (times 5, 15 and 30 min.) with equal volumes of heperinized saline reinfused. The plasma levels of NE and E were determined by radio-enzymatic analysis and plasma C by radioimmunoassay. Some animals were selected for blood sampling through the catheter and by decapitation to compare C values. The concentrations of NE, E, C, and the area under the curve ( AUC ) for the stress response time course were compared using a two-tailed student's t-test ($P<.05$). The levels of NE, E, and C at 5, 15, and 30 min. were compared to baseline, and catheter vs. decapitated C levels, by the paired t-test ($P<.05$). The correlation coefficients for all parameter pairings were calculated and since this was a large number of pairings only those reaching $P<.001$ were considered significant.

C levels were lower in blood obtained by catheter as compared to decapitation. Individual rats responded differently to immobilization and females usually showed higher stress values. NE and E levels rose smoothly and simultaneously in all animals and peaked early. The early (5 min) NE and E levels, but not the baseline (0 min), predicted ($P<.001$) the total extent of NE and E stress responses. C levels were independent of NE and E, dropping initially (5 min) in most animals and rising to peak at the end of immobilization (30 min). Supported by United States Public Health Service Grant AA 06017.

372.4 BIOGENIC AMINES IN BRAIN AND ADRENALS OF RATS WHICH CAN OR CANNOT COPE WITH STRESSFUL FOOTSHOCK. M. Graffy Sparrow* and W.H. Vogel. Department of Pharmacology, Thomas Jefferson Univ., Philadelphia, PA 19107.

It is generally accepted that stressful events cause stress which can manifest itself as changes in various biochemical measures, such as central and peripheral biogenic amine levels and turnover. Recent studies from this and other laboratories seem to indicate that it is not the exposure to a stressor, but the ability or inability of the animal to control the stressor, which causes most of these changes. To explore the effects of coping and noncoping on the stress response, we exposed pairs of rats to identical footshock (FS) for either a single one hour trial or for one hour on each of days 1 and 3. One animal was given the ability to terminate the shock (coping) while the yoked animal had no control over the shock (noncoping). A third rat remained unstressed in the home cage. Biogenic amine profiles of adrenals and various brain areas were studied. Although adrenal weights decreased significantly in noncoping rats exposed to one hour of FS, no changes in norepinephrine (NE), epinephrine, or dopamine (DA) were seen in adrenals in either study. Significantly lower NE levels were measured in the frontal cortex (FC) of noncoping rats in both groups and in the thalamus of coping rats only after two FS trials. DA levels rose significantly in the pons-medulla (PM) of noncoping animals after one trial and of coping rats after both single and double trials, and in the hypothalamus (HT) of coping and the striatum of coping and noncoping rats after one hour FS. Furthermore, the ratios of DA to its metabolite dihydroxyphenylacetic acid were significantly lower in the FC of noncoping animals of both exposure groups and of coping animals after one trial. 5-hydroxyindole acetic acid (5-HIAA) levels increased significantly after one hour FS in the FC of both coping and noncoping animals and in the FC and PM of noncoping animals after two exposures. Serotonin/5-HIAA ratios were significantly higher in the HT of coping rats and significantly lower in the PM of noncoping rats after two exposures. These results clearly show that the biochemical events detected are not only due to the FS schedule but also to the ability or inability of animals to cope with a stressor.

372.5 DEPENDENCE OF GLUCOCORTICOID ACTIONS IN HIPPOCAMPUS MODULATING VIP-STIMULATED CYCLIC AMP FORMATION ON IN VIVO PRESENCE OF ACTH. Allan Harrelson* and Bruce S. McEwen. Laboratory of Neuroendocrinology, Rockefeller University, New York, NY 10021

Glucocorticoids (GC's) are known to exert effects on a variety of brain functions and biochemical mechanisms. In particular, it has been reported by Mobley and Sulser (Nature 286:608 (1980)) that GC's inhibit norepinephrine (NE)-stimulated cyclic AMP (cAMP) formation in slices from rat frontal cortex. Subsequent studies from the same laboratory (J. Pharm. Exp. Therap. 226:71 (1983)) showed that this GC action was equally effective in adrenalectomized (ADX) and hypophysectomized (HYPOX) rats, implying that GC's act directly on the brain, without the mediation of pituitary hormones such as adrenocorticotropic hormone (ACTH). We have recently discovered that ADX increases, and in vivo GC replacement suppresses, the stimulation of cAMP in hippocampal (HIPP) slices by vasoactive intestinal peptide (VIP) in ADX rats (cf Harrelson et al, Neurosci. Abstr. 9:26.13 (1983)). The time course is approximately two days and is consistent with a genomic mechanism of GC action on some component of adenylate cyclase (AC) activation. In the present study, we investigated whether GC regulation of AC in the HIPP was also independent of the pituitary. In our first experiment, we gave implants of the synthetic GC, dexamethasone (DEX), to HYPOX rats and measured VIP-stimulated cAMP accumulation in HIPP slices relative to stimulation in HYPOX controls. The DEX replacement was ineffective, implying that pituitary hormones, and not GC's, might be regulating AC. A similar result was obtained for NE-stimulated cAMP formation in HIPP from HYPOX rats, contrasting with the suppression obtained with DEX in ADX rat HIPP. In the second experiment, VIP-stimulated cAMP accumulation was found to be increased by HYPOX, just as by ADX. Administration of ACTH to HYPOX animals reduced VIP-stimulated cAMP levels, just as DEX treatment had done in ADX rats. However, when we gave ACTH to HYPOX rats which had been ADX, we found that there was no response, implying that the presence of adrenal gland is necessary for ACTH to have its effect. Taken together with other findings that gonadal hormone and mineralocorticoid treatments have no effect, these results suggest that GC's are the primary modulators of VIP-stimulated AC in the HIPP, but that ACTH may be permissive for such regulation. Such an interpretation is consistent with other studies which have indicated that ACTH may have specific effects on HIPP electrophysiology and biochemistry.

372.6 EARLY, ENVIRONMENTAL FACTORS MODIFY THE DEVELOPMENT OF HIPPOCAMPAL GLUCOCORTICOID RECEPTORS AND THE ADRENOCORTICAL STRESS RESPONSE. M.J. Meaney, D.H. Aitken*, S. Bhatnagar*, S.R. Bodnoff* and L.J. Iny*. Center for Studies in Behavioral Neurobiology, Concordia University, Montreal, Canada H3G 1M8.

In previous work (Behav. Neurosci., in press) we reported that early, postnatal handling alters the development of the glucocorticoid receptor (Gr) system in selected brain regions. The handling procedure involves the daily separation of pups from the nest and their mother for 15 min. As adults, animals handled from Days 1 to 21 showed significantly higher (≈35 to 40%) Gr levels (Bmax) in hippocampus and frontal cortex than did nonhandled animals (measured using an in vitro receptor binding assay with (3H)dexamethasone as ligand). There were no differences found in the hypothalamus, pituitary, amygdala, or septum, nor were there any differences in the affinity (Kd) for (3H)dexamethasone. In the work reported here we have found that the handling effect on Gr development is apparent by Day 7 of life, and that the magnitude of the effect at that time is indistinguishable from that seen in adult animals. Moreover, when examined as adults, animals handled from Days 1 to 7 or from Days 8 to 14 did not differ from animals handled from Days 1 to 21 in hippocampal Gr levels and each of these three groups differed significantly from animals handled from Days 15 to 21 and nonhandled animals. Thus, the sensitivity of the Gr system to the effects of handling wanes between the second and third weeks of life, a period that corresponds to the development of adult Gr levels in the hippocampus (Meaney, Sapolsky & McEwen, Dev. Brain Res., 18:165, 1985). In subsequent work we found that, compared to handled animals, nonhandled animals as adults hypersecrete corticosterone following the termination of a stressor (i.e., exhibit a significantly longer period of elevated corticosterone following stress) suggesting a less efficient adrenocortical negative-feedback system in the nonhandled animals. Sapolsky, Krey and McEwen (Proc. Nat. Acad. Sci. USA, 81:6174, 1984) have presented strong evidence for a relationship between hippocampal Gr levels and adrenocortical negative-feedback and our findings are consistent with their results. Taken together, these findings suggest that early, environmental factors may permanently modify the adrenocortical stress response and that this effect appears to be mediated through the development of the hippocampal Gr system.
(Supported by Medical Research Council grant MA-8632)

372.7 FEAR INDUCED CARDIAC ARRHYTHMIAS IN THE DIGITALIS PREDISPOSED RABBIT: AMYGDALOID CENTRAL NUCLEUS CONTRIBUTIONS. C.G. Markgraf,* B.S. Kapp and C.D. Khazam.* (SPON. J. van Houten). Dept. of Psychology, Univ. of Vermont, Burlington, Vt. 05405.

Clinical observations suggest that intense emotional states such as fear can induce potentially lethal ventricular arrhythmias, particularly in a diseased myocardium inclined to electrical instability (Natelson, 1979). Accordingly, animal models have been developed to more accurately define the conditions under which such emotional states generate these arrhythmias (Lown & Verrier, 1978; Natelson et al., 1978; Skinner et al., 1975). Lacking, however, is the application of an appropriate animal model to the identification of the central nervous system (CNS) circuitry participating in emotionally induced arrhythmias. The present study was therefore designed to establish such an appropriate model, and to apply it to an identification of the CNS circuitry contributing to the production of emotionally induced arrhythmias.

In Experiment I, 18 New Zealand rabbits received Pavlovian fear conditioning trials in which a tone conditioned stimulus (CS) was paired with a shock unconditioned stimulus (US). A retention test was given 24 hours later during which ouabain, a digitalis glycoside, was infused i.v. (110 µg/kg) over a 50 min period to predispose the heart to electrical instability. Presentations of the CS alone during infusion resulted in a significant increase in arrhythmic episodes when compared to the pre-CS baseline periods ($p < .05$). These CS-induced arrhythmias were preceded by bradycardia and included ventricular premature contractions, ventricular tachycardia, and complex ventricular ectopic beats, as well as idiojunctional rhythms and various types of heart block. An additional experiment demonstrated that they were significantly reduced by atropine methylnitrate ($p < .01$), thereby indicating a parasympathetic involvement in their generation.

Previous research (Kapp et al., 1984) has suggested that the amygdaloid central nucleus (ACE) contributes to the expression of bradycardia to a CS during Pavlovian fear conditioning. Experiment II was therefore conducted to determine if electrical stimulation of the ACE, which produces bradycardia, would induce arrhythmias in the digitalis-predisposed rabbit. Four rabbits received Pavlovian fear conditioning followed 24 hours later by ouabain infusion and random presentations of either the tone CS or electrical stimulation of the ACE (60-80 µA), both 5.0 sec in duration. Both CS presentation and stimulation produced arrhythmias of similar topography, and in both cases they were preceded by bradycardia and blocked by atropine methylnitrate.

The results demonstrate an animal model with which to investigate emotionally induced arrhythmias, and suggest a contribution for the ACE in the generation of parasympathetically-mediated arrhythmias. Supported by the American Heart Association.

372.8 ROLE OF ALPHA1- AND BETA-ADRENERGIC RECEPTORS IN THE CARDIOVASCULAR RESPONSES SHOWN TO CLASSICALLY CONDITIONED DEFENSE REACTION IN THE RAT. M.F. Callahan, R.F. Kirby, M.J. Brody and A.K. Johnson. Depts. of Pharmacology and Psychology and The Cardiovascular Center. The University of Iowa, Iowa City, IA 52242.

The current studies examined the role of the sympathetic nervous system mechanisms mediating the hemodynamic responses to conditioned aversive environmental stimuli. Male Sprague-Dawley rats received miniaturized pulsed Doppler flow probes on the left renal (REN) and superior mesenteric (MES) arteries and the lower abdominal aorta (Hq) for the determination of blood flow and carotid and jugular catheters for the determination of blood pressure and drug infusions, respectively. Following a recovery period, rats received 30 conditioning trials/day. The conditioned stimulus (CS) consisted of a light/tone (15 sec), the termination of which initiated an unconditioned stimulus of intermittent foot shock (1mA, 15 sec). On successive days, animals received pharmacological antagonism of adrenergic systems between trials 10 and 11 and the responses of these trials were compared. The hemodynamic responses to the CS consisted of a primary pressor response occurring at approximately 1 sec (~13 mmHg) followed by a smaller secondary pressor response (~7 mmHg) for the duration of the CS. At 1 sec post CS onset, there was a significant MES vasoconstriction with no change in the other beds. At 8 sec post CS onset, MES resistance reached a peak while the Hq bed showed a significant decrease. Following $alpha_1$-adrenergic receptor blockade with prazosin (0.5 mg/kg, i.v.), the pressor response and the MES vasoconstriction were attenuated. In addition, the Hq vasodilation appeared to be attenuated. Beta-adrenergic receptor blockade with dl-propranolol (0.1 mg/kg, i.v.) blocked the Hq vasodilation seen at the 8 sec time point, leading to a larger pressor response (7 mmHg before-vs-13 mmHg after propranolol). The results of these studies demonstrate that the cardiovascular response to a conditioned aversive stimulus consists of a complex hemodynamic pattern. The primary pressor response at 1 sec post CS onset is mediated by increased sympathetic drive, reflected in part by increased mesenteric vasoconstriction. The secondary pressor response is mediated in large part by the interaction of mesenteric vasoconstriction and skeletal muscle vasodilation. These mesenteric and skeletal musle responses are mediated by $alpha_1$- and beta-adrenergic receptors respectively.

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372.9 COMPARISON OF THE ACTIONS OF REPEATED STRESS AND REPEATED ANTIDEPRESSANT TREATMENT ON RAT BRAIN NORADRENERGIC RECEPTORS AS ASSESSED BY CATECHOLAMINE-STIMULATED cAMP FORMATION IN BRAIN SLICES. E.A. STONE, J.E. PLATT and A.S. HERRERA*. Dept. Psychiatry, New York Univ. Sch. of Med., New York, NY 10016.

Previous studies have shown that repeated stress and repeated antidepressant treatment both reduce the function of postsynaptic noradrenergic receptors in the rat brain as evidenced by a reduced cAMP response to NE in brain slices. The latter response is known to be mediated by a beta adrenergic receptor which is directly linked to adenylate cyclase and an alpha adrenergic receptor which modulates the size of the beta response. The present studies were undertaken to determine which of these receptors is affected by stress and antidepressants.

Restraint stress was administered repeatedly to rats twice daily for 10 days. The antidepressant drug, desmethylimipramine, (DMI) was injected 10 mg/kg, ip, twice daily for 10 days. Animals were killed 24 hrs after treatment. Cerebral cortical slices were incubated with catecholamine agonists and antagonists and assayed for cAMP accumulation by published methods. In order to selectively activate alpha adrenergic receptors, slices were incubated either with 6-fluoro-NE (6-FNE) alone or NE in the presence of timolol. To selectively activate beta receptors either isoproterenol (ISO) alone or NE plus phentolamine or prazosin was employed. To activate alpha and beta receptors either NE alone or 6-FNE plus ISO was used.

Restraint stress was found to have no effect on the cAMP response to either pure alpha or pure beta adrenergic stimulation but to significantly reduce the response to combined alpha and beta stimulation. DMI was found to have no effect on the response to alpha stimulation but to reduce significantly the responses to both beta and alpha plus beta stimulation. The decrease in the alpha plus beta response after DMI was attributable entirely to the reduction of the beta component of the response.

The results suggest that restraint stress and DMI affect the brain catecholamine cAMP generating system at two different loci. DMI acts selectively to reduce the function of beta receptors whereas stress acts selectively to reduce the synergistic interaction between alpha and beta stimulation. Since stress has been previously reported to alter some properties of brain beta adrenergic receptors however it is possible that the reduced alpha-beta interaction results from some change in the beta receptor which is not manifested in the ISO-cAMP response.

Since they appear to act at different sites, antidepressants and stress may be capable of enhancing the subsensitivity to NE caused by each other. If confirmed this may constitute a mechanism by which antidepressants could enhance adaptation to stress by magnifying the latter's effect on the reduction of the NE-cAMP response. (Supported by grants MH22768 and MH08618).

372.10 AN ESTRADIOL-INDUCED PROTEIN SYNTHESIZED IN THE VENTRAL MEDIAL HYPOTHALAMUS (VMH) AND TRANSPORTED TO THE MIDBRAIN CENTRAL GRAY (MCG). C.V. Mobbs, R.E. Harlan, and D. W. Pfaff (SPON: Z.M. Wenzel). The Rockefeller University, New York, NY 10021.

Several lines of evidence suggest that the estradiol (E2)-dependent female sexual behavior, lordosis, is regulated in part by the synthesis of proteins in the VMH which are transported to the MCG. We developed a strategy involving microinfusion of radioactive amino acids, followed by 2-dimensional gel electrophoresis, to identify proteins which may be involved in this system. A mixture of 35-S-methionine and 35-S-cysteine (2:1, total 500-1000 uCi), suspended in 1 uL phosphate buffered saline, is infused bilaterally into the VMH over a period of 2 hours into female rats which have been ovariectomized at least one week and given silastic implants containing E2 at the beginning of infusion. The rats are sacrificed either 6 or 14 hours after receiving E2, and 16 brain regions are obtained by microdissection. Samples are analyzed by 2-dimensional gel electrophoresis, entailing isolectric focusing in the first dimension and SDS-polyacrylamide electrophoresis (molecular weight separation) in the second dimension, followed by visualization by fluorography, routinely separating at least 250 spots. The infusion procedure results in the incorporation of at least 4 million cpm into TCA-precipitable protein in the VMH; about 5% of this amount is usually found in the MCG samples. In turn, the MCG routinely contains 5-10 times more cpm than regions immediately adjacent, which have fewer projections from the hypothalamus. We consistently find a protein spot with MWr of 70 kilodaltons, migrating on the basic side of the gel, which appears very rarely in VMH samples from ovariectomized rats given no E2 replacement, but almost always in rats given E2 replacement. This protein spot is similarly induced in the MCG samples. Three lines of evidence suggest that the labelled protein in the MCG is due to transport from the VMH and not local synthesis: (1) radioactivity after infusion is much higher in regions with hypothalamic projections than other regions; (2) tubulin, which is synthesized at high levels in the VMH but is transported slowly, is much less abundant in the MCG samples; (3) previous studies using a similar infusion procedure followed by autoradiography have directly visualized radioactivity in axonal projections to the MCG but not in cell bodies in the MCG. It is not clear if the protein is synthesized de novo in response to E2, or is the result of a modification of another protein. In any case, these studies suggest that E2 causes the appearance of this protein in the VMH, from whence it is transported to the MCG. The possible roles this protein may play in mediating functional effects of E2 remain to be established. (Supported by NIH grants AG05326 and HD05751).

372.11 STRESSOR CONTROLLABILITY AND PITUITARY-ADRENAL ACTIVITY. N. H. Kalin*, C. M. Barksdale*, S. M. Ryan and S. F. Maier. W. S. Middleton Memorial V.A. Hospital, Dept. of Psychiatry, Univ. of Wisconsin, Madison, WI 57305, and Dept. of Psychology, Univ. of Colorado, Boulder, CO 80309.

The degree of behavioral control which an organism has over a stressor can modulate the behavioral and physiological impact of that stressor. Rats exposed to a single session of 80 inescapable and unavoidable (uncontrollable) shocks reveal a variety of subsequent behavioral and physiological changes not seen following exposure to a series of physically identical escapable (controllable) shocks. It has often been argued that uncontrollable aversive events are more "stressful" than controllable aversive events and that some component of the stress response might mediate many or all of the behavioral and physiological changes. Virtually all of these changes are influenced by pituitary-adrenal hormones, and differential release of these hormones might be intimately involved in the mediation of these stressor controllability effects. However, pituitary-adrenal hormones have not been measured in controllability experiments which use the same species and shock parameters as those used to demonstrate the differential behavioral and physiological outcomes. Thus here we measured plasma ACTH and corticosterone levels after shock conditions identical to those used in the behavioral controllability experiments. Rats were given either 80 escapable, yoked inescapable, or no shocks. Plasma ACTH and corticosterone were measured by radioimmunoassay either immediately, 30 min, 60 min, 150 min, or 24 hr following the shock treatment. There were no differences in either the ACTH or corticosterone response to the escapable and inescapable shock. Many of the effects of controllability are typically assessed 24 hr following shock treatment and involve reexposure to the stressor. It is still possible that the pituitary-adrenal response of escapably and inescapably shocked subjects to subsequently occurring stressors might differ. We thus exposed escapably shocked, inescapably shocked, or non-shocked subjects to 5 footshocks 24 hr after initial treatment. ACTH and corticosterone were measured immediately, 30 min, and 60 min after the shock reexposure. Again, there were no differences between escapably and inescapably shocked subjects. Thus differential levels of ACTH and corticosterone cannot be used to explain behavioral and physiological differences produced by these shock treatments.

372.12 INESCAPABLE TAILSHOCK STRESS POTENTIATES THE INHIBITORY EFFECTS OF MUSCIMOL ON NEURONAL FIRING IN RATS SUBSTANTIA NIGRA PARS RETICULATA. Daniel W. Hommer, Robert C. Drugan, Gene R. Stoner*, Jacqueline N. Crawley, and Steven M. Paul*. Clinical Neuroscience Branch, NIMH, NIH, Bethesda, MD 20205.

Stress may affect neuronal inhibitory systems which employ gamma-aminobutyric acid (GABA) as a neurotransmitter. Inescabable tailshock stress has been reported to facilitiate bicuculline-induced seizures 2 hours after the stress (Drugan et al., 1985, Brain Research, in press). This facilitatory effect of inescapable shock on seizures can be prevented by pretreatment with aminooxyacetic acid, an agent which elevates GABA levels. (McIntyre et al., 1984, Neurosci. Abst.). Enhancement of the effects of the GABA agonist muscimol on GABA release in striatal slices following cold and immobilization stresss also has been reported (Kuriyama et al., J. Neurochem 42: 943-950, 1984). These findings suggest that stress may potentiate the actions of GABAergic agents.

The substantia nigra pars reticulata (SNR) is heavily innervated by GABAergic fibers and may be involved in the GABAergic modulation of seizure activity. We investigated the effect of inescapable shock stress on the ability of muscimol to inhibit neuronal firing in the SNR.

Male Sprague-Dawley rats were pretreated with either no shock, or 80 five second tailshocks (increment from 1-2 mA) at a rate of 2 per minute. Two hours after the last shock rats were anesthetized with chloral hydrate and mounted in a stereotaxic apparatus. A burr hole was drilled over the SNR and a single barrel microelectrode lowered into the brain until a unit which had extracellularly recorded characteristics matching those previously identified with SNR neurons was found. After a five minute baseline was recorded muscimol was administered at two minute intervals in doses of 1, 1, 2, 4 and 8 mg/kg i.v.

Muscimol produced a significant, dose dependent decrease in the activity of SNR neurons both in the shock-stressed (n=13) well as in the unstressed animals (n=13). However, the stressed animals were significantly more sensitive to muscimol-induced inhibition than control animals (3-way ANOVA with repeated measures on the dose; $F(1, 24) = 5.1$, $p < .03$).

This data suggest that stress may produce a functional neuronal supersensitivity to GABAergic agents. The precise nature of this supersensitivity remains to be elucidated.

372.PO *PSEUDOCHOLINESTERASE ACTIVITY IN PATIENTS WITH MITRAL VALVE PROLAPSE, W.A. Price, A.J. Giannini, D.A. Hoffman, R.H. Loiselle. Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272.

Both elevated pseudocholinesterase (PC) levels and mitral valve prolapse (MVP) are known to occur with increased frequency in patients with anxiety disorders, panic attacks and agoraphobia. Pseudocholinesterase is felt to be a biological marker for trait anxiety, while MVP is thought to be associated with hypernoradrenergic tone. To date no relationship is known to exist between PC and MVP. PC levels were obtained, using the Dupont (ACA) clinical analyzer method, from 15 patients (10 women, 5 men; ages 22-65) with the Axis I diagnosis of anxiety disorder, panic attacks or agoraphobia, as determined using DSM-III criteria. These patients additionally had confirmed MVP as determined by both cardiac ausculatory findings and echocardiogram. These levels were compared against 15 similar sex and age matched controls who had MVP, but no associated psychiatric diagnosis. Results revealed that only 2 of the 15 patients with some form of anxiety disorder and MVP had elevated PC levels, which compared exactly to the number encountered in the control group. There therefore does not seem to be a relationship between MVP and trait anxiety as measured by elevated PC levels.

## MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: CORTEX

373.1 INTERCONNECTIONS AMONG CORTICAL MOTOR OUTPUT REGIONS (CMORs) CONTROLLING THE FACIAL MUSCULATURE OF CAT. R.S. Waters, D.P. Friedman, M. Bornschlegl. The Rockefeller University, Laboratory of Neurophysiology, New York, N.Y. 10021 and Neurosciences Research Branch, NIDA and Laboratory of Neuropsychology, NIMH, Bethesda, MD 20205.

We previously investigated the pattern of brainstem projections to the facial musculature of cat from independent low threshold cortical motor output regions (CMORs) in the border between motor and premotor cortex (areas 4,6), suprasylvian gyrus (SII), parietal cortex (SIII), and cortex along the medial bank of the anterior ectosylvian sulcus (SIV). While the CMORs do not project directly to the motor neurons of the facial nucleus (FN), they do project to several intermediate relay sites in the brainstem where facial motor integration may occur prior to reaching FN. While the motor output from each CMOR appears to be independent of the other CMORs, available physiological evidence suggests that at least some of the CMORs may be interconnected at the cortical level and may thus serve to modulate motor output. In order, therefore, to understand the pattern of connectivity among these regions we examined connections in the CMORs using a combination of electrophysiological mapping and anatomical tracing techniques. Injections of tritiated amino acids or HRP were made into physiological identified sites in each CMOR which yielded contractions of facial muscles with low current microstimulation. The following results were obtained:

Injection of tritiated leucine-proline into area 4-6 border resulted in terminal labeling in SII, SIII, and SIV. Isotope injection into SII produced labeling in area 4-6, SIII and SIV, whereas SIII injections yielded labeling only in area 4-6 and SIV. SIV injections produced labeled cells in area 4-6, SII and SIII although the possibility exists that isotope may have spread into SII. These results demonstrate the existence of reciprocal connections between all pairs of related fields except between SII and SIII, where a projection to SII could not be positively identified. Labeling appeared densest and most extensive in layer I.

The results demonstrate the existence of extensive interconnections among the CMORs. Each CMOR in itself exerts independent motor control over facial musculature and yet has access to a variety of thalamo-cortical and cortico-cortical input which may serve as a closed loop system to modulate or update its own motor output. The CMORs represent an interactive set of independent parallel descending motor systems that may form the anatomical substrate for voluntary control of complex facial movements.

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373.2 THALAMO-CORTICAL INPUT TO A PHYSIOLOGICALLY IDENTIFIED CORTICAL MOTOR OUTPUT REGION (CMOR) IN PARIETAL CORTEX, AREA 5, CONTROLLING THE FACIAL MUSCULATURE OF CAT. M. Bornschlegl* and R.S. Waters (SPON: D. Dahl) The Rockefeller University, Laboratory of Neurophysiology, New York, NY 10021.

The cortical area in cats immediately posterior to the ansate sulcus, area 5, is described as the third somatosensory cortex. Neurons in this area receive somatotopically organized input from the contralateral side of the body. This region contains a relatively large face representation in its most lateral part. Intracortical microstimulation (ICMS) delivered into the lateral part of area 5 elicits contraction of facial muscles with stimulating currents as low as 2-3 uA. In addition, the motor effect from this cortical motor output region (CMOR) is independent of the integrity of the motor cortex. In this study, by using a combination of electrophysiological and anatomical techniques we investigated the thalamo-cortical projection to area 5 to determine whether, this CMOR receives thalamic input characteristic of other known cortical motor regions.

Cats were anesthetized with Halothane gas, and a chamber was installed over the parietal cortex. Following surgery cats were maintained on low doses of Ketamine (1-2mg/kg) throughout the experiments. A tungsten-in-glass microelectrode was inserted into area 5, and the face region was identified using the microstimulation technique (trains of 10-12 cathodal pulses, 0.2ms duration, 300 Hz, 30uA maximum intensity). A micropipette filled with a 20% HRP solution in 0.2M KCl was then driven into the center of the physiologically identified facial motor region, and HRP was iontophoresed. After a 48 hr survival time, the cat was sacrificed and the tissue was processed according to the TMB method.

Examination of tissue sectioned frontally through the thalamus revealed two locations of heavy retrograde labeling. One was found in the medial part of the posterior nuclear complex (POm), where it forms a shell dorsally over the ventrobasal complex. This labeling in POm was continuous with labeling in the caudal parts of VL, where it borders on VB. The other location was found in the rostro lateral parts of the VA-VL complex. Only occasionally did labeled cells appear in VB and in the small-celled part of CL and LP. While the thalamocortical projection from POm may mediate a variety of sensory input to area 5, the connections between area 5 and the lateral parts of VA-VL, known as thalamic relay for cerebello-cortical projections, give further support to the interpretation of area 5 as a sensorimotor area.

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373.3 RELATIONS BETWEEN DIRECTION OF 3-DIMENSIONAL ARM MOVEMENTS AND SINGLE CELL DISCHARGE IN PRIMATE MOTOR CORTEX. A.B. Schwartz, R. Kettner, and A.P. Georgopoulos. Bard Laboratories of Neurophysiology, Department of Neuroscience, The Johns Hopkins University, School of Medicine, Baltimore, MD 21205.

How is the discharge of single cells in the motor cortex related to the direction of 3-D arm movements? Previous studies using 2-D arm movements coupled to an articulated manipulandum have shown that the activity of single motor cortical cells is broadly tuned to the direction of movement (Georgopoulos et al., J. Neurosci. 2:1527, 1982). Can this finding be generalized to 3-D, unconstrained arm movements? We studied this problem by recording the activity of single cells in the motor cortex while monkeys moved their arm and touched lighted targets in a 3-D space in front of them in a reaction time task. The targets were arranged as the corners of a cube with 15cm edges. Movements were made in 8 directions from the center of the cube to each of the targets. Thus, the spherical extrapersonal space was sampled at equal angular intervals. The center of the cube was 22 cm in front of the midline of the animal's body and at shoulder level. The trajectories of the movements were monitored using a 3-D ultrasonic tracking system (J.G. Chubbuck and A.P. Georgopoulos, Soc. Neurosci. Abst. 10:337, 1984). We found that motor cortical cells related to arm movements are, indeed, broadly tuned to the direction of movement in 3-D space. For a particular cell, the frequency of discharge is highest in association with movements in a certain direction (preferred direction) and decreases in an orderly fashion with movements made farther away from the preferred direction. Preferred directions differ among different cells. Preliminary analyses suggest that the cell's 3-D "tuning volume" can be described by Fisher's distribution on the sphere.

These results indicate that the directional tuning is a general property of cells in the motor cortex. The presence of a preferred direction and broad tuning suggest that the coding of the direction of movement may rely on the neuronal population, along the lines suggested previously ("vector hypothesis", Georgopoulos et al., Exp. Brain Res. Suppl. 7:327, 1983). (Supported by USPHS Grants NS17413, NS07226, MH18030.)

373.4 RESPONSE OF NEURONS IN MONKEY MOTOR CORTEX TO STOPPING LIMB MOVEMENTS. E. V. Evarts, V. A. Jennings* and S. P. Wise. Laboratory of Neurophysiology, NIMH, Bethesda, MD 20205

If, as Phillips hypothesized in 1969, certain somatosensory inputs to the primary motor cortex (MI) during active movements signal deviations from an intended movement, then the magnitude of MI neuronal responses should be graded according to the degree of mismatch between actual and intended displacement. This possibility was examined by recording the response of MI neurons to stopping wrist movements in two operantly conditioned rhesus monkeys. The monkeys made wrist flexion-extension movements of 15° in the paradigm described elsewhere (Jennings, this volume). In task A, stops always occurred at the same angular position (the "stop position"), but the angular distance to the target (the "remaining distance") varied. In task B, the remaining distance was held constant at 12°, but the stop position was varied.

Neurons over a wide area of the forelimb region of MI responded to stopping movements with increases or decreases in activity at latencies of 20 ms to 65 ms. The present analysis focuses on neurons in rostral regions of MI that showed phasic bursts of activity before the onset of movement in one (the "preferred") direction. These neurons responded to stops of movements in the preferred direction with sustained increases in activity. In general, neurons with the largest stop responses also had the largest premovement phasic burst of activity. In task A, these MI neurons showed a graded stop response that was proportional to the magnitude of the remaining distance. The neuronal stop response increased by approximately 11 IPS/deg as the remaining distance increased from 1.5° to 13.5°. At the maximum remaining distance tested (13.5°), i.e., when the stop position was closest to the start zone, the magnitude of the stop response was similar to the magnitude of the phasic burst of premovement activity. In task B, when the stop position was shifted in the preferred movement direction with the remaining distance held constant, the stop response increased by about 3 IPS/deg.

It is concluded that the stop response of these MI neurons depends on the distance remaining to be moved and the joint position at which the stop occurred. The graded nature of this response and its resemblance to the muscle stop response (see Jennings, this volume) support the hypothesis that MI neurons contribute to mechanisms that act to compensate for deviations from an intended movement. Further, the similarity of the stop response (at the maximum remaining distance) to the premovement activity burst suggests that MI makes a comparable contribution to motor activity at the beginning of movement and the resumption of a stopped movement.

373.5 REPRESENTATION OF MOVEMENT DIRECTION IN PRIMATE MOTOR CORTEX IN THE ABSENCE OF IMMEDIATE MOVEMENT. M.D. Crutcher, A.B. Schwartz, and A.P. Georgopoulos. Bard Laboratories of Neurophysiology, Department of Neuroscience, The Johns Hopkins University, School of Medicine, Baltimore, MD 21205.

Visual targets were presented to monkeys who were trained to withhold their movement toward the target. A two-dimensional working surface and an articulated manipulandum were used. The animals grasped the manipulandum and captured first a light at the center of the plane. After a variable period of time, the center light was turned off and another light was turned on at one of several positions on a circle of 8 cm radius. A variable delay (range = 0.5-3.2s) was then imposed during which the animals did not move but waited holding the manipulandum at the center of the plane. At the end of the delay period the target light dimmed. This served as a "go" signal to capture the target. Thus movements of different directions were generated after the delay, but the information about target location and upcoming movement direction was present with the first appearance of the target.

The activity of 120 motor cortical cells that were related to contralateral arm movements and to task performance was recorded in 3 hemispheres of 2 monkeys. Data were analyzed for 6 different target locations for each cell (N = 6x120 = 720). Statistically significant changes in neuronal activity during 0.5s following the initial presentation of the target were detected in 476/720 (66%) of the cases. Increases in activity were observed twice as frequently as decreases. The shortest latency changes were observed at the 40-60ms bin, and 50% of them had occurred by 100ms following target onset. Many cells showed changes in activity that extended throughout the delay period. These changes usually differred according to the direction of the upcoming movement. Similar findings were obtained when the initial position of the hand was at the periphery and the final point at the center of the plane. Does this early recruitment of motor cortical cells result in a substantial change of firing in the neuronal population? Population histograms were constructed for each direction of the upcoming movement after the spike trains of single trials were aligned to target onset. A significant increase (3 to 4 fold) in the population activity was observed 80ms after the target onset. Do these changes predict what the upcoming direction of movement will be? A directional analysis of the population changes was performed, according to the "vector hypothesis" (Georgopoulos et al., Exp. Brain Res. Suppl. 7:327, 1983). We found that indeed the direction of the population vector during the delay period was close to the direction of the movement that was to be made 0.5-3.2s later. These results suggest that the motor cortex might be involved in early processing of the upcoming movement direction, even in the absence of immediate motor output. (Supported by USPHS Grants NS17413, MH15330, NS07226).

373.6 DIFFERENCES IN THE SPATIAL RELATION BETWEEN MOVEMENT DIRECTION-DEPENDENT AND LOAD DIRECTION-DEPENDENT ACTIVITY CHANGES IN PRIMATE CORTEX AREAS 4 & 5. J.F.Kalaska, D.A.D.Cohen* and M.L.Hyde*. Centre de recherche en sciences neurologiques, Faculté de médecine, Université de Montréal, Québec, Canada, H3C 3J7.

We reported previously (Neurosci.Abstr.10:738) that the activity of motor cortex area 4 cells in a 2-dimensional reaching task shows large continuously-graded changes for both the direction of arm movement and the direction of external loads applied to the arm. We also reported that only the first parameter produces large changes in the activity of parietal area 5 neurons. Area 5 cells show much smaller changes in their movement-related activity than do area 4 cells while a monkey compensates for loads applied in different directions. Thus, the activity of single area 5 cells during motor behavior does not reflect the muscle contractile activity to the same degree as does that of area 4 cells.

In both regions, the changes in a cell's activity related to different directions of movement were centred on one direction, its "preferred direction" for movement. Similarly, changes in activity caused by loads applied to the limb in different directions were centred on one direction, or "load axis". We describe here an important difference in the spatial relationship between these two properties of each cell for the two cortical regions.

In area 4, the preferred direction and the load axis for each cell tended to be opposite (mean difference 179° ± 49°). A cell's activity in this task may be represented by a vector oriented along its preferred direction (Exp.Br.Res.Suppl.7:327). We will show that the vectorial sum of the load direction-dependent changes in the activity of the area 4 cells in our sample is a vector oriented in a direction approximately opposite to that of the applied load. Thus, the net area 4 population response to an applied load is a signal to apply a force in a direction opposing the load. These data for shoulder-related cells is consistent with previous experiments for cells related to more distal arm movements.

In contrast, there does not appear to be a fixed relationship between the preferred direction and the load axis for cells in area 5. As a result, the small load-dependent activity changes seen at the single-cell level in our area 5 sample are apparently further reduced at the population level, because these variations tend to cancel out when the cell activities are added vectorially. This implies that the overall pattern of activity of the area 5 population should provide a rather reliable and unambiguous signal about the posture and movement of the limb in space, that does not vary with changes in the relative degree of contractile activity of the muscles involved, unlike area 4. The data suggest that this feature of area 5 activity results from mechanisms that opperate at both the single-cell and population levels. (Supported by an MRC Scholarship and MRC grant M-7693).

373.7 GABA-ERGIC INHIBITION IN THE PRIMATE MOTOR CORTEX AND THE CONTROL OF VOLUNTARY MOVEMENT. M.Matsumura*, T. Sawaguchi* and K.Kubota. Dept. of Neurophysiology, Primate Res. Inst., Kyoto Univ., Inuyama, Aichi 484, Japan.

Effects of intra-cortical injection of GABA, its agonist (Muscimol) and antagonist (Bicuculline) into the hand motor area were studied, at behavioral and single neuron levels, while the monkeys were performing a visual reaction time task. Monkeys pressed or released the lever by hand in response to a visual cue.

After injection of Muscimol (2 -6 μg), using Hamilton syringe, into the hand motor area, identified by intra-cortical micro-stimulation, the reaction time for lever release became significantly longer, and the amount of task-related EMG activities of the forearm and upper arms decreased. These effects began to appear 5 min. after the injection and lasted for more than 30 min.. After a similar injection of Bicuculline (10 -20 μg), the variability of the reaction time increased, (S.D. more than two times greater than control condition), and EMG durations became longer. Further, EMG activities, which showed reciprocal patterns under the control condition, became bi-directionally active. In later stages after injection, twitching appeared in muscles during inactive phases in the control, and finally convulsion occurred.

GABA, Mucimol and Bicuculline were iontophoretically applied to isolated single neurons, of which activities were related to lever press and/or lever release. Ninety-seven neurons, including 14 PTNs, of the hand area of the contralateral motor cortex in four macaque monkeys, were related uni- or bi-directionally to the lever press or release movement. In 90% of 45 neurons showed an activity decrease to GABA and/or Muscimol applications (current<30nA), and in 90% of 85 neurons Bicuculline showed an activity increase at lever release or lever press phases without any changes in background activity. In seven neurons, Bicuculline induced uni-directional discharge patterns into bi-directional ones. These results suggest that GABAergic inhibitory neurons play a role in forming spatially and temporally organized reciprocal discharge patterns in the motor cortex neurons and that their activities, in turn, lead to reciprocally organized hand movement.

373.8 CORTICO-CORTICAL INPUTS TO IDENTIFIED MOTOR CORTEX NEURONS. P. Zarzecki. Department of Physiology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

Cortico-cortical neurons synapse upon pyramidally-shaped neurons. However, the cells of origin of many corticofugal pathways are pyramidally-shaped, so that this does not specify which of these receive cortico-cortical contacts. Electrophysiological studies have revealed cortico-cortical inputs to pyramidal tract neurons (Blum et al. 1968, Exp. Neurol.), but the occurrence of monosynaptic cortico-cortical epsps outside of layer V of cortex (Herman et al. 1985, Exp. Brain Res.) indicates that cortico-cortical effects are not restricted to PT neurons. Accordingly, in this study we tested a variety of antidromically identified cortical neurons for cortico-cortical inputs. In barbiturate anaesthetized cats, electrodes were stereotactically placed to antidromically activate cortical neurons projecting to the striatum, thalamus, red nucleus, and through the cerebral peduncle. Optimum placement of stimulating electrodes was aided by depth-threshold analyses and confirmed histologically. This report is based upon 70 antidromically identified neurons of the motor cortex. Current spread calculations and branching analyses confirmed that projections to separate subcortical targets usually originate from separate cortical neurons. Cortico-cortical effects were evoked by surface stimulation of area 3a of primary somatosensory cortex and were detected by collision-extinction techniques and, when subthreshold or inhibitory, by the facilitation or retardation of antidromic invasion which they cause.

Excitatory cortico-cortical effects have been detected upon cortico-thalamic, cortico-rubral, and cortico-peduncular neurons. The latency of cortico-cortical excitation ranged from 0.9 to 4.1 ms. Inhibition was always later. In recording tracts where cortico-cortical excitation has been observed, it has never appeared upon all corticofugal neurons. The strongest evidence for this apparent selectivity in the distribution of cortico-cortical excitation was obtained when several neurons of the same corticofugal class were recorded and studied simultaneously. In all 8 instances, cortico-cortical effects could be detected for only some of the nearby neurons projecting to the same sub-cortical target. Furthermore, thalamo-cortical effects were distributed mostly, but not exclusively, to neurons without detectable cortico-cortical input. In conclusion, excitation from somatosensory cortex to motor cortex reaches cortico-thalamic and cortico-rubral neurons, in addition to pyramidal tract neurons, but it seems not to be distributed equally to all members within a class of corticofugal neuron.
Supported by the MRC of Canada.

373.9 THE ORGANIZATION OF INTERCONNECTIONS BETWEEN THE PREMOTOR AREAS IN THE PRIMATE FRONTAL LOBE AND THE ARM AREA OF PRIMARY MOTOR CORTEX. D.C. Primrose* and P.L. Strick. V.A. Med. Ctr. and Depts. of Neurosurg. and Physiol., SUNY-Upstate, Syracuse, NY 13210.

In macaques, the premotor areas which project most densely to the arm area of the primary motor cortex are: 1) the arcuate premotor area (APA), located in and around the caudal bank of the arcuate sulcus and 2) the supplementary motor area (SMA), located on the medial wall of the hemisphere (Muakkassa and Strick, Brain Res. '79). In the present experiments we examined the topographic distribution, laminar origin and laminar termination of projections from the premotor areas to the arm area of the primate motor cortex using amino acid and WGA-HRP transport.

The "arm areas" of the APA and SMA have substantial interconnections with one another and with "arm" regions of the primary motor cortex on the precentral gyrus. On the other hand, the two premotor areas do not have significant interconnections with more caudal regions of motor cortex in the deeper two-thirds of the central sulcus. This topographic pattern suggests that the APA and SMA are functionally related to the "rostral zone" of arm representation in primary motor cortex, but not to the "caudal zone".

Interconnections between primary motor cortex, APA and SMA originate from cells located in the same cortical layers: largely layer III, but also layers II and V. However, the laminar patterns of termination in the 3 cortical areas differ. Efferents from the primary motor cortex terminate in a trilaminar pattern in the premotor areas. One band of labeling is located in layer I, another in layer III and a third in layer V. The most dense terminations in the premotor areas are located in layer III. In contrast, efferents from the premotor areas terminate in a bilaminar pattern in the motor cortex. One band of labeling is located in layers I, II and the outer one half of layer III, and the other band is in layer VI. The most dense terminations in the motor cortex are located in layer I. Based on these patterns of termination, the projections from the motor cortex to the premotor areas are similar to the "forward" cortico-cortical connections of sensory systems, while the projections from the premotor areas to the motor cortex are similar to the "backward" connections (e.g., Maunsell and Van Essen, J. Neurosci. '83). These observations raise significant questions about the classical schemes of hierarchical organization proposed for frontal motor areas.

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373.10 MULTIPLE AND DIFFERENTIAL PROJECTIONS FROM THE PARIETAL LOBE TO THE PREMOTOR AREAS OF THE PRIMATE. D.D. Galyon* and P.L. Strick (SPON: D.Hoffman). V.A. Med. Ctr. and Depts. of Neurosurg. and Physiol., SUNY-Upstate, Syracuse, NY 13210.

There is extensive clinical and experimental evidence for the involvement of the parietal lobe in the generation and control of voluntary movement. Classically, the influence of the parietal lobe has been thought to be mediated via its projections to "premotor areas" in the frontal lobe. Using retrograde transport of WGA-HRP, we have examined the connections of the parietal lobe with each of the "arm areas" in the two premotor areas which project most heavily upon the macaque motor cortex, i.e., the arcuate premotor area (APA) and the supplementary motor area (SMA) (Muakkassa and Strick, Brain Res.'79).

The arm areas of both the APA and the SMA receive substantial projections from regions considered to be part of the classical somatosensory areas (SI, SII, and SIII). The APA receives projections only from part of the SII arm representation within the lateral fissure. In contrast, the SMA receives projections from part of the SI arm representation in area 2 just lateral to the postcentral sulcus and from part of SIII in area 5 on the medial wall of the hemisphere.

The intraparietal sulcus is the source of substantial projections to the arm areas of the APA and SMA. The APA receives projections from a part of area 5 located deeply in the sulcus, from part of area 2 located laterally in the sulcus and from part of area 7 located laterally in the sulcus and extending onto the adjacent cortical surface. In contrast, the SMA receives projections from separate medial and lateral regions of area 5 located superficially in the sulcus and extending onto the cortical surface.

The arm areas of the APA and SMA also receive projections from different parts of lateral area 7. The APA receives a projection from more rostral parts of area 7 located just dorsal to SII on the cortical surface. The SMA receives projections from caudal parts of area 7B located in the lateral fissure and on the adjacent cortical surface.

In total, there are 12 spatially separate cortical regions in the parietal lobe which project to the "arm areas" of the APA and SMA. Five of these parietal regions project to the APA and 7 to the SMA. Surprisingly, none of the parietal regions project to both premotor areas. These findings suggest that there are multiple parietal regions concerned with the control of arm movement and that these parietal regions distinguish between the APA and SMA.

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373.11 SINGLE UNIT ACTIVITY IN THE PARIETAL CORTEX OF THE PRIMATE DURING A HAPTIC DELAYED MATCHING-TO-SAMPLE TASK. Kevin W. Koch and Joaquin M. Fuster. Neuroscience Interdepartmental Ph.D. Program, Brain Research Institute and Dept. of Psychiatry, University of California, Los Angeles, CA 90024.

Anatomical and physiological data suggest that the parietal association cortex serves in the complex somesthetic discriminations that take place during the manual exploration of the environment. In order to test this idea, the present study analyzes neuronal activity in the parietal lobe of behaving monkeys during a task requiring the perception and temporary retention of haptic (stereognostic) information. Extracellular records of 300 single units in parietal cortex of 2 rhesus monkeys were taken during a haptic delayed matching-to-sample (DMS) task. In a task trial the monkey, sitting in a dimly lit and sound attenuated chamber, first hears a warning click, indicating that the sample object, either a cube or a sphere (26 mm diameter/side), is in position to be manipulated. The animal then reaches through an opening to where he has learned he will find the object, which is attached to the upper end of a vertical rod and in a central position (the same for all trials) in front of the animal. After the animal has felt and pulled it, the sample object is withdrawn and an 18-sec delay period initiated. At the end of the delay, another click signals the presentation of the choice objects: a cube and a sphere, 10 cm apart. The animal has 3 sec to feel both objects and choose the correct one (matching the sample) by giving it a slight pull, for which he is rewarded with a squirt of fruit juice. The sample object and the side (right or left) of the correct choice are varied randomly from trial to trial. Preliminary data analysis indicates the following: (1) the majority of cells in all areas examined underwent significant changes in firing during the presentation of the sample object; those changes were usually related to the arm projection and/or the manipulation of the object, more rarely to the warning click; (2) in anterior areas, firing changes were generally excitatory, while in posterior areas most of the cells were inhibited during sample presentation; (3) cells in posterior areas were likely to show changes during the delay and those changes were usually inhibitory; (4) fewer delay-related cells were found in anterior positions, although their delay changes were excitatory; (5) a small proportion of the cells showing increased activity during the haptic manipulation or delay period were differentially activated by the cube and the sphere. The results indicate that neurons in parietal cortex participate in the sensory-motor processes of manual exploration and perception of objects. Some neurons in anterior parietal cortex appear involved in the discrimination and short-term retention of haptic sensory attributes. (Supported by NSF Grant BNS-8213806)

373.12 EFFERENT TOPOGRAPHY OF THE MACAQUE SUPPLEMENTARY MOTOR AREA: DEMONSTRATION WITH INTRACORTICAL STIMULATION. A. R. Mitz. Laboratory of Neurophysiology, NIMH, Bethesda, MD 20205.

The supplementary motor area (SMA), like the primary motor cortex (PMC), has a direct corticospinal projection (Murray and Coulter, J. Comp. Neurol., 195:339, 1981). However, examination of SMA efferent organization with standard microstimulation has been hampered by the paucity of evoked movements, contributing to the recent suggestion that the SMA may lack a topographic organization (Macpherson et al., Exp. Brain Res., 45:410, 1982).

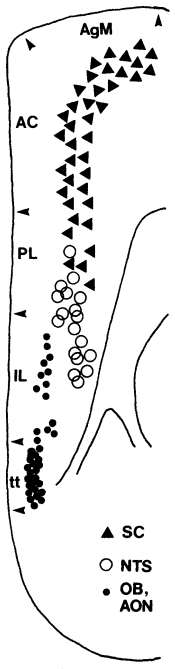
Two rhesus monkeys were implanted with chronic recording chambers over the left SMA and glass-coated platinum-iridium electrodes were inserted into the SMA through the dura. Three methodological changes greatly increased the number of movements evoked from the SMA: (1) The electrode-tip surface area was increased from 100-250 $um^2$ to approximately 1000 $um^2$. (2) 100 ms pulse trains were used because they were significantly more effective than pulse trains under 50 ms. (Other stimulation parameters were: 0.2 ms/phase biphasic pulses, 330 pulses/second, currents usually below 60 ua.) (3) Because SMA responses were more labile than PMC responses, and apparently more easily masked by an uncooperative animal, the number of responses was increased by delivering stimuli at nonuniform intervals or by sedating the animal with 1.3 mg/kg of ketamine.

Orofacial movements, including movements of the pinnae, lips, tongue, and jaw, and conjugate eye movements to the contralateral visual hemifield, were observed most rostrally. Contralateral forelimb movements, including movements of the digits, wrist, forearm, elbow, and shoulder, were evoked from sites immediately caudal to the face area. Hindlimb and tail movement sites were caudal to the forelimb representation, however, preliminary analysis was unable to establish a clear boundary between hindlimb sites of the primary motor cortex and hindlimb sites of the SMA. The orofacial, forelimb, and hindlimb representations each spanned from the dorsal bank of the cingulate sulcus, to the medial surface of the hemisphere and at least 2 mm onto the hemispheric convexity. Movements of the neck and trunk were evoked from 3 or 4 apparently separate areas in the convexity and medial wall of the hemisphere. The overall rostrocaudal extent of the SMA covered 12-14 mm.

373.13 EFFERENT PROJECTIONS FROM THE MEDIAL FRONTAL CORTEX TO THE NUCLEUS TRACTUS SOLITARIUS, SUPERIOR COLLICULUS AND OLFACTORY BULB. E.J. Neafsey, Karen Hurley-Gius* and Dimitrios Arvanitis*. Departments of Anatomy and Pathology, Loyola University Medical Center, Maywood, IL 60153.

The rat medial frontal cortex (MFC) projects to the nucleus tractus solitarius (NTS; Terreberry and Neafsey, BR:278, 1983), the superior colliculus (SC; Wyss and Sripanidkulchai, BR:293, 1984), and the olfactory bulb (OB; Haberly and Price, JCN:181, 1978). Using double retrograde labeling methods we have attempted to determine the topography and amount of overlap between the MFC cell populations projecting to these targets.

Thirteen male rats (350-580g) were utilized in these experiments. Each animal was anesthetized with ketamine HCl (100mg/kg, IP) and had his cisterna magna opened to permit access to the NTS. All animals received an injection (0.4ul, 2%) of fluorescent tracer (Fast Blue, Diamadino Yellow or Rhodamine microspheres) just lateral to the obex into the region of the NTS and a second injection of a different tracer into either the ipsilateral OB (n=9) or the ipsilateral SC (n=4). All animals were recovered from surgery and the OB/NTS group was allowed to survive 4-5 days, while the SC/NTS group survived 7-8 days. Each animal was then sacrificed, and the tissue processed for visualizing fluorescent labeling.




As the figure to the right illustrates, the pattern of retrograde labeling is topographic. Cells projecting to the OB and anterior olfactory nucleus (filled circles) are located most ventrally in the taenia tecta (tt) and in the more superficial layers of the infralimbic cortex (IL). Cells projecting to the NTS (open circles) are located in the deep layers of IL and in prelimbic cortex (PL), with the most ventral of these being overlapped by more superficially located OB projecting cells. Interspersed among the NTS cells in PL are cells which project to the SC. These cells continue dorsally thru the anterior cingulate (AC) into medial agranular cortex (AgM).

This anatomical arrangement of the efferents from the MFC is consistent with the functional division of the MFC into a ventral "visceral motor" cortex and a more dorsal frontal eye field area. Since behaviors such as feeding, reproduction, foraging and territorial marking require the integration of olfactory, gustatory, visceral and visual information, it is likely that the MFC is part of the cortical sensorimotor apparatus involved in these behaviors.

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373.14 CHANGES IN CORTICOMOTONEURONAL (CM) POSTSPIKE FACILITATION OF EMG ACTIVITY ASSOCIATED WITH LOGARITHMIC AND SQUARE TRANSFORMATION OF THE EMG SIGNAL. P.D. Cheney and K. Mewes*. Dept. of Physiol., Univ. of Kansas Med. Ctr., Kansas City, KS 66103.

Although spike-triggered averaging of EMG activity is a powerful method of establishing causal relations between cortical cell discharge and muscle activity in awake monkeys, it suffers from the low yield of cells that produce clear postspike facilitation (PSF) (Fetz, E.E. and Cheney, P.D., J. Neurophysiol. 44:751, 1980). One factor that could contribute to the low yield of clear PSF is the possibility that some cortical cells preferentially facilitate only a fraction of the recorded motor units, for example, only the small or large units (Clough, J.F.M., et al., J. Physiol. 198:145, 1968). We therefore, attempted to enhance PSF by selectively increasing the amplitude of small motor unit potentials relative to large ones using a logarithmic (log) amplifier and large potentials relative to small ones using an amplifier which squared the EMG signal. Multichannel, spike-triggered averages of the simple rectified EMG signal as well as log and square transformed versions of the same rectified EMG signal were computed simultaneously. We tested a sample of 10 CM cells yielding a total of 67 spike-triggered averages, 42 of which showed PSF. We used two types of measures to quantitatively compare the magnitude of PSF in averages of different versions of the EMG signal. With one measure, the mean increase of PSF above baseline was expressed as a percent of baseline. The averages of percent mean PSF in the simple rectified, log and square versions of the EMG signal from 42 facilitated muscles were 4.7%, 2.8% and 9.6%, respectively. From these results, squaring the EMG signal seems to produce a marked enhancement of PSF and the log transformed EMG, a marked reduction in PSF. However, these changes were due largely to changes in the average baseline EMG signal rather than to genuine effects on the strength of PSF. To avoid this problem, we quantified PSF with a signal-to-noise measure in which the increase above baseline of the mean PSF was divided by the standard deviation of the baseline points. Based on this mean-to-noise measure, 15 of 42 PSFs were enhanced an average of 20% in the log transformed signal and 11 of 42 PSFs were enhanced an average of 25.3% in the squared EMG signal. However, log or square transformation revealed no PSF from CM cells that was not evident in the average of simple rectified EMG signals nor did it identify any additional CM cells. We conclude that log and square transformation of EMG signals can yield modest improvement of PSF, although generally not sufficient in magnitude to warrant routine use of these procedures. The results also emphasize the limitations of expressing the magnitude of postspike effects as a percent of baseline. We suggest that a more consistent and accurate indicator of the strength of postspike effects is provided by signal-to-noise measures. Supported by NSF grant BNS 82-16608.

373.15 MOTOR CORTEX RESPONSES TO CEREBELLAR STIMULATION IN THE MONKEY. S. Pullman, R. L. Watts and E. V. Evarts. Laboratory of Neurophysiology, NIMH, Bethesda, MD 20205

Neuronal responses to electrical stimulation of the brachium conjunctivum were recorded in primary motor cortex of monkeys in which pyramidal tract neurons (PTNs) were identified by their antidromic responses to electrical stimulation of the medullary pyramid. The major goals of the study were to determine (1) the responsiveness of motor cortex PTNs as compared to more superficially situated motor cortex neurons and (2) the responsiveness of PTNs as a function of axonal conduction velocity. Recordings were obtained from awake, chronically prepared monkeys that sat relaxed during recordings and that had not been trained to execute specific movements.

Data were obtained on 494 non-PTNs and 158 PTNs in the primary motor cortex. With 200 usec stimuli of 0.3 to 0.5 mA, 141 (29%) of the non-PTNs were activated, with the response commonly being a single spike with a latency of about 3 msec. Increasing the stimulus to 1.0 or 1.5 mA sometimes evoked two successive spikes with latencies of 3 and 6-8 msec. The shortest response latency was 2.5 msec. The cerebellar-evoked cortical field potential had a latency of 1.6 msec, and showed a sign reversal at about 1.5 mm below the cortical surface, the same depth at which non-PTN cerebellar responses were most prominent. Marking lesions made via the microelectrodes during recording of the most prominent responses were found to be in cortical layer III upon subsequent histological analysis.

Of the 158 PTNs that were recorded, 5 (3.2%) showed responses to cerebellar stimulation. Even in these 5 PTNs the average numbers of spikes per stimulus were less than in non-PTNs; latencies ranged from 3 to 10 msec. Because only 5 PTNs were activated, these data could not answer the question as to the correlation between PTN axonal conduction velocity and responsivesness to cerebellar inputs.

In conclusion, it was found that reponses to stimulation of the brachium conjunctivum were approximately ten times more prominent in cells of cortical layer III than in PTNs. The greater prevalence of cerebellar-evoked unit responses in layer III is consistent with anatomic data showing a concentration of thalamic terminal arborizations in layer III. While this does not contradict previous anatomic and physiologic evidence as to the existence of monosynaptic thalamic inputs to PTNs (all of which are in layer V), the present results show the relatively greater strength of the inputs to layer III neurons under the conditions of relaxed immobility that prevailed in the present study.

373.16 CONTRASTING FEATURES OF SOMATOSENSORY RESPONSES IN PYRAMIDAL TRACT NEURONS (PTNs) AND NON-PTNs IN SENSORIMOTOR CORTEX OF THE MONKEY. R. L. Watts, S. Pullman, and E. V. Evarts. Laboratory of Neurophysiology, NIMH, Bethesda, MD 20205.

Previous studies contrasting the responses of pyramidal tract neurons (PTNs) and non-PTNs in precentral and postcentral sensorimotor cortex of monkeys have been carried out during volitional movements and actively maintained postures. In such studies the relatively small numbers of PTNs that were systematically investigated made it difficult to compare quantitative features of sensory responses in PTNs (all of which are located in layer V) and more superficial neurons. This study, which was carried out in awake monkeys that had not been trained to perform movements and that sat quietly during recordings, allowed collection of larger samples of neurons and revealed significant differences in the somatosensory responses of PTNs and more superficial cells in both primary motor (MI) and in primary sensory (SI) cortex.

Recordings were concentrated in a rostral region of MI that received deep inputs generated by wrist displacement and in a region of area 2 in SI that was likewise activated by wrist displacement. Ramp flexor and extensor displacements of up to 15° and of 50 msec duration were produced by a DC torque motor. In both MI and SI, responses were more prominent in superficial neurons than in PTNs. Of a total of 226 non-PTNs recorded in MI, 78 (35%) were activated by wrist displacement whereas only 7 (12%) of 56 PTNs in MI were responsive to wrist displacement. In SI, 260 (74%) of 352 non-PTNs were responsive to wrist displacement and 9 (40%) of 22 PTNs responded.

For the 78 responsive MI non-PTNs, 74 of the responses were of the phasic or mixed phasic/tonic type (median response latency (MRL) 16 msec) and 4 responses were of the purely tonic type (MRL 87.5 msec). For the 7 responsive MI PTNs, 5 responses were of the phasic or mixed type (MRL 26 msec) and 2 were of the tonic type (MRL 64 msec). In SI, the responses of the 260 non-PTNs consisted of 239 that were phasic or mixed (MRL 16 msec) and 21 that were tonic (MRL 60 msec). Of the 9 SI PTN responses, 8 were phasic or mixed (MRL 20 msec) and 1 was tonic (latency 100 msec).

These findings parallel results on differences between cerebellar-evoked responses in PTNs as compared to more superficial MI neurons and show that PTNs are relatively less responsive to somatosensory as well as to cerebellar inputs, at least under conditions of relaxed immobility.

373.17 COURSE AND TERMINATIONS OF THE RODENT CORTICOSPINAL TRACT FROM PHYSIOLOGICALLY IDENTIFIED AREAS OF SENSORY AND MOTOR CORTEX. C.F. SIEVERT AND E.J. NEAFSEY. Dept. of Anat., Loyola Univ. Med. Cntr., Maywood, Il 60153.

Using WGA-HRP as an anterograde tracer, the course and termination of the rodent corticospinal tract (CST) from various physiologically identified areas of sensory and motor cortex were studied in twenty-eight male Long Evans rats (300-500 grams). Animals were anesthetized with Ketamine HCL (100 mg/kg IM) and placed in a stereotaxic apparatus. The cisterna magna was opened to prevent cortical swelling, and a craniotomy performed over the left frontal and parietal cortex. Forelimb primary and secondary somatosensory areas were delineated by recording evoked multiunit activity during peripheral stimulation. Motor areas were identified and mapped by intracortical microstimulation with a glass insulated tungsten electrode. Stimulation parameters were 0.25 msec pulses, 350 hz, 300 msec trains; currents were limited to 50 uamps. After mapping, small injections (one per animal) of wheat germ agglutinin-HRP were made in the rostral forelimb motor area, caudal forelimb motor area, forelimb primary sensory area, hindlimb sensory-motor area and the secondary somatosensory area. Animals were perfused and the tissue processed for HRP histochemistry using TMB. Spinal cord sections were cut in the coronal plane to best visualize terminal labeling, and in the horizontal plane to best demonstrate fibers. Corticospinal fibers were found in five areas of the cord, including both dorsal funiculi, both lateral funiculi and the ipsilateral ventral funiculus. All five paths could be found as low as the lumbar enlargement in animals which received injections in the hindlimb area. The heaviest "terminal labeling" was found contralaterally but there was also some ipsilaterally in most animals. The forelimb primary sensory cortex had heavy terminations to the medial portions of lamina III-VI in the dorsal horn. Secondary somatosensory cortex had terminations similar to but lighter than the forelimb primary sensory cortex, the major difference being the lack of ipsilateral terminations. Hindlimb sensory-motor cortex had terminations at lumbar levels in lamina II-VII with sparse terminations in lamina VII. The caudal forelimb motor cortex had terminations more ventrally in lamina IV-IX with very sparse terminations in lamina VIII. Finally, the rostral forelimb area had terminations similar to the caudal forelimb motor area, except that lamina VIII in the medial ventral horn appeared to contain significant terminal labeling.

(Supported by NIH grant NS 16146).

373.18 AN ULTRASTRUCTURAL AND MORPHOMETRIC STUDY OF UNMYELINATED AXONS IN THE PYRAMIDAL TRACT OF MONKEYS. A.P. Thomas* (SPON: K. Chan). Dept of Anatomy, School of Medicine, Univ. of Puerto Rico, San Juan, P.R. 00936

The pyramidal tracts of fascicularis and rhesus monkeys were used in this study. The pyramidal tracts (PT) were prepared for light and electron microscopy (Langford and Coggeshall, Anat. Rec. 197: 297, 1980). 1mm-thick cross-sectional areas from the medullary pyramid were divided into three or four small blocks. One-half micron thick sections from each of the blocks were studied with the light microscope. The total cross-sectional area of the PT was obtained from thick sections. Ultrathin sections (70 nm thick) cut from each of the blocks were double stained with uranyl acetate and lead citrate. These sections were scanned and systematically examined with a transmission electron microscope. Photographs were taken at regular intervals.

Unmyelinated axons were identified based on the presence of microtubules, neurofilaments, and mitochondria that differentiated them from glial processes. Unmyelinated axons were observed to be scattered among the myelinated axons. Unmyelinated axons occurred: singly; in small groups of two or six; or in large groups of twenty to twenty-five. Diameters of unmyelinated axons were measured; 200 randomly-selected axons were categorized as small, medium, or large in diameter and the various diameters within each group were averaged. Small-diameter axons ranged from 0.1 to 0.224 μm; mediums ranged from 0.225 to 0.424 μm; and large diameters ranged from 0.425 to 0.9 μm. Unmyelinated axons made up about 13% of the total population of PT axons. Components of unmyelinated axons observed among the young, were also observed in mature adult fascicularis and rhesus monkeys. The number of myelinated axons per PT ranged from 490163 to 583920. Thus, electron microscopy has demonstrated the occurrence of a significant number of unmyelinated axons in the PT of fascicularis and rhesus monkeys. (SUPPORTED by NIH grants RR00166 and NS16934).

373.19 ORIGINS OF THE CORTICOSPINAL TRACT. R. J. Nudo* and R. B. MASTERTON. Department of Psychology, Florida State University, Tallahassee, FL 32306. (SPON: John A. Jane).

To visualize the cells of origin of the corticospinal tract, a hemisection of the spinal cord was performed just above C2 and flakes of HRP placed in the cut. A very high degree, if not complete backfilling of the corticospinal tract was obtained by this procedure in 22 selected species of mammals: The vividness of the pyramidal decussation, the number of labeled cells in the brainstem tegmentum, red nucleus, etc., each provide independent assurance that few descending fibers might have escaped labeling. At neocortex, Golgi-like labeling of neuronal somata was invariably obtained.

Although a full comparative analysis is still underway, certain generalizations can already be made. First, the CST in many mammals takes origin in a much wider area of neocortex than previously supposed. Second, all corticospinal neurons originate in layer V. No exception to this generalization has yet been found. Third, the total number of CST cells in neocortex rises sharply across phyletic grades based on the animal's propinquity with mankind. This marked increase in number is attained first by a stacking of the CST cells, then by a reduction in the stacking but a concurrent expansion of the total area containing CST cells and an increase in their average size. Fourth, animals at higher levels of the sequence show an increasing number of "new" areas of neocortex containing concentrations of CST cells.

This acquisition of what might be considered "new" corticospinal tracts, together with the cytoarchitectural changes in the number, size, distribution, density and arrangement of cells within each cortical field, each independent of more general morphological features such as body size, would seem to be of physiological as well as evolutionary significance. (Supported by NINCDS NS07726.)

## BEHAVIORAL PHARMACOLOGY: PHENCYCLIDINE AND OPIATES

374.1 BEHAVIORAL AND BIOCHEMICAL EFFECTS OF PHENCYCLIDINE IN DIFFERENTIALLY HOUSED MICE. C.A. Wilmot, C. VanderWende and M.T. Spoerlein. Rutgers University, Dept. Pharmacology, PO Box 789, Piscataway NJ 08854

The abuse of phencyclidine (PCP) is noted for producing psychoses and unpredictable episodes of violent behavior. The following studies were conducted to determine (1) whether acute or chronic treatment with PCP would modify the fighting behavior of grouped (GH) or individually housed (IH) mice, (2) the relationship between the effects of PCP on motor activity and fighting and (3) the effects of PCP on the accumulations of 5-HTP and DOPA after decarboxylase inhibition in GH and IH mice.

PCP, 0.625-2.50 mg/kg, produced a dose-dependent increase in the total time spent fighting in mice individually housed from 10 to 45 days, but did not increase the percentage of mice fighting at any time. These doses did not increase motor activity, suggesting some behavioral selectivity of low doses for fighting behavior. The effects of PCP on fighting were dependent on the condition of IH, as neither acute nor chronic treatments of PCP to GH mice produced fighting. IH mice had significantly greater accumulations of DOPA and higher levels of tyrosine in the striatum and frontal cortex compared to GH mice. Significant effects of PCP treatment on 5-HTP and DOPA accumulations were seen in the striatum and frontal cortex of IH mice only.

In conclusion, PCP facilitated the expression of fighting behavior in IH but not GH mice at doses that did not stimulate motor activity. A difference in the functional activity of monoaminergic systems may be the basis of the increased sensitivity of IH mice to the behavioral effects of PCP.

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374.2 THE EFFECTS OF DSP-4 ADMINISTRATION ON PHENCYCLIDINE-INDUCED LOCOMOTOR AND EATING BEHAVIORS IN THE RAT. P.A. Seymour and R.G. Browne, Central Research Division, Pfizer Inc., Groton, CT 06340.

A large body of evidence implicates a role for norepinephrine (NE) in locomotor and consummatory behaviors in the rat. Administration of the psychotomimetic agent, phencyclidine (PCP), has been shown to induce pronounced increases in horizontal locomotor activity (Castellani and Adams, Eur. J. Pharmacol., 73:143, 1981) and eating (unpublished observations). It has been suggested that PCP acts, in part, by a presynaptic mechanism involving the release of norepinephrine from noradrenergic terminals (Marwaha et al., J. Pharmacol. Exp. Ther., 215:3, 606, 1980), and by enhancing the release of dopamine from terminals in the striatum (Vickroy and Johnson, J. Pharmacol. Exp. Ther., 223:669, 1982). The noradrenergic-selective neurotoxin, DSP-4, has been shown to markedly deplete NE in the CNS, with maximal reductions occurring within 6 hours after administration (Jonsson et al., Eur. J. Pharmacol., 72:173, 1981). To examine the effect of acute NE depletion on PCP-induced locomotor and eating behaviors, male CD rats were injected with DSP-4 (50 mg/kg, i.p.) or vehicle and 6 hours later were placed into computer-monitored behavioral chambers for the assessment of baseline behavior. Twenty-four hours later PCP (3.2 mg/kg, s.c.) was administered and data were collected for an additional 24 hours. DSP-4 administration severely reduced baseline eating and locomotor activity, as measured by both horizontal (crossovers) and vertical (rearing) data. Pretreatment with DSP-4 significantly attenuated the PCP-induced increases in crossovers and eating, although significant increases in these behaviors were observed. It was concluded, therefore, that NE plays a major role in normal locomotor activity and eating, but may only play a minor role in the PCP-induced manifestations of these same behaviors.

374.3 EFFECTS OF CALCIUM ANTAGONISTS ON PCP AND AMPHETAMINE-INDUCED LOCOMOTOR STIMULATION IN MICE. Ronald G. Browne and Patricia A. Seymour. Pfizer Inc., Central Research, Groton, CT 06340.

Several lines of recent evidence suggest that calcium channel blockers might be useful as psychotherapeutic drugs. In animals, calcium antagonists displace PCP from high affinity binding sites (Eldefrawi et al. Biochem. Pharmacol., 31:2549, 1982.), and, at high doses, antagonize PCP-induced stereotyped behavior in rats (Shah et al., Pharmacologist, 24:160, 1982). A more recent link between calcium and antipsychotic drugs was the demonstration (Proc. Natl. Acad. Sci. 80:5122, 1983) that some antipsychotic drugs act as calcium channel antagonists. In order to explore the possibility that various calcium channel blockers might be active in tests predictive of antipsychotic activity, we examined the effects of these agents on amphetamine- or PCP-induced locomotor stimulation in mice. Male CD1 mice (at least 8/group) were injected with vehicle or test compound 15 min before receiving 3.2 mg/kg of d-amphetamine or 3.2 mg/kg of PCP. Fifteen min after the challenge drug, the mice were placed into individual chambers designed to record locomotor activity. The effect of various calcium blockers on amphetamine-induced locomotor stimulation revealed that at least 10 mg/kg of drug was necessary to reduce amphetamine's effect. However, at these doses, the drugs alone frequently produced a sedative effect. Neither Bepridil nor Lidoflazine had a significant effect on amphetamine hyperactivity even at 100 mg/kg. PCP at 3.2 mg/kg also increased locomotor activity. Verapamil dose-relatedly reduced PCP hyperactivity, but the dose necessary to produce this effect was no different from that required to reduce amphetamine-induced activity. Consistent with the effects seen with amphetamine, neither bepridil nor lidoflazine antagonized PCP. However, low doses (3.2 mg/kg) of nitrendipine, nimodipine or nifedipine significantly reduced PCP-induced stimulation, in contrast to the lack of effect at this dose against amphetamine-induced hyperactivity. Furthermore, a dose of 3.2 mg/kg of these calcium blockers given alone did not significantly reduce locomotor activity. Therefore, the observed anti-PCP activity cannot be explained as a superimposed sedative effect. Furthermore, pharmacokinetic studies indicate that the calcium antagonists do not produce their effects by altering the disposition of PCP. The results of these experiments showing that various calcium channel blockers antagonize PCP-induce hyperactivity at doses which, by themselves, are non-sedating, suggests that these agents may be selective PCP antagonists.

374.4 EFFECT OF DOSE OF CYCLAZOCINE ON PHENCYCLIDINE-INDUCED LOCOMOTOR AND STEREOTYPED BEHAVIOR IN MICE. M.E. Nevins. Dept. of Biological Research, G.D. Searle & Co., Skokie, Ill. 60077

The behavioral effects of and the interaction between two drugs that bind with high affinity to the phencyclidine receptor, phencyclidine HCl(PCP) and the opiate mixed agonist-antagonist cyclazocine, were studied. The effects of i.p. administration of PCP and cyclazocine on locomotor and stereotyped activity of mice were studied both alone and in combination. Locomotor activity and stereotyped activity were assessed by the use of microprocessor-based infra-red photocell activity monitors. Locomotor activity is expressed as the accumulated distance the mouse travels. Stereotypy is defined here as any repetitive movements that interrupt any photocell beam repeatedly and is expressed as accumulated time spent in repetitive behaviors.

PCP doses of 5, 10 and 20 mg/kg increased both locomotor and stereotyped activity. Evidence of ataxia was seen in the dose-related delay of peak locomotor and stereotypic effects at the two higher doses. Cyclazocine doses of 0.5 and 2 mg/kg had no significant effect on either total locomotor or total stereotyped activity accumulated over the 60 minute test. At 10 mg/kg cyclazocine significantly increased both locomotor activity ($p<.05$) and stereotypy ($p<.05$).

The effects of the interaction of cyclazocine and PCP on locomotor and stereotyped activity were studied by pretreating mice with either 0, 0.5, 2, 10 or 25 mg/kg of cyclazocine 20 minutes prior to administration of PCP 5 mg/kg. Pretreatment with 0.5 mg/kg of cyclazocine significantly reduced the total locomotor ($p<.05$) and the peak (20-40 minutes post PCP injection) stereotypy ($p<.05$) effects of PCP. Pretreatment with 2 mg/kg of cyclazocine had no effect on PCP-induced locomotion or stereotypy. The two higher doses of cyclazocine, 10 and 25 mg/kg, both significantly delayed the peak locomotor ($p<.05$) and the peak stereotypic ($p<.05$) effects of PCP. This delay in peak effect is similar to that induced by higher doses of PCP. The results are discussed in terms of a possible dose-related effect of cyclazocine on PCP with low doses of cyclazocine partially antagonizing PCP effects and higher doses adding to PCP effects.

374.5 STRONG ABSOLUTE CONDITIONED PLACE PREFERENCE IS OBTAINED WHEN MORPHINE IS PAIRED EXCLUSIVELY WITH TACTILE STIMULI IN AN OPEN FIELD. P. Vezina and J. Stewart. Center for Studies in Behavioral Neurobiology, Concordia University, Montreal, Quebec, Canada H3G 1M8.

Despite the widespread use of conditioned place preference techniques (CPP) to assess the reinforcing properties of drugs of abuse little is known about the basis of this effect. Although it has generally been assumed that stimuli associated with morphine come to elicit approach and continue to do so in the absence of the drug empirical support for this view has been equivocal prompting some to question the validity of the technique.

Three groups of rats were trained and tested in an open field (68x68 cm). The conditioned stimuli (CS) used were entirely tactile (since these require an animal to make direct contact with them in order to experience them) and consisted of different floor textures in the open field. Care was taken to minimize the influence of other stimulus modalities. The open field floor was divided into four quadrants. For conditioning, all four quadrants were of the same texture (either wire screen or steel rod). For testing, the number of quadrants having the floor texture previously paired with morphine (CS+ quadrant) was reduced and combined with quadrants of the texture previously paired with saline (CS- quadrant).

During conditioning, one group received morphine (10 mg/kg, i.p.) paired with the wire screen floor on one day and saline paired with the steel rod floor on the following day. A second group received the opposite pairings and a third control group received saline with both floor textures. This procedure was repeated four times. Conditioning sessions lasted 30 min. On three subsequent test days all animals were administered saline and placed in the open field with different numbers of CS+ quadrants available (1, 2 or 4 quadrants). Time spent on the CS+ quadrant as well as horizontal locomotion throughout the open field were measured for 30 min.

Both conditioning groups spent from 66-85% of their time on the CS+ when two CS+ quadrants were present. When only one CS+ quadrant was present, animals still demonstrated a CPP for the CS+ of 64-74% (chance on this test was 25%). This preference was observed through out the test sessions. No preference for any particular quadrant was evident when all four quadrants were CS+. Control group animals demonstrated a slight preference for the screen floor. It was also found that animals from conditioning groups were least active when only one CS+ quadrant was present, intermediately active when only two CS+ quadrants were present, and most active when all four quadrants were CS+. Thus, the fewer the number of CS+ quadrants, the lower the activity. Hence, once an animal made contact with the CS+ it maintained contact with the stimulus and reduced its approach to and contact with other stimuli. These CPP and locomotion data clearly demonstrate that stimuli previously associated with morphine subsequently elicit approach and contact in the absence of the drug.

374.6 MAGNETIC FIELD INHIBITION OF MORPHINE-INDUCED ANALGESIA: POSSIBLE INVOLVEMENT OF CALCIUM AND OTHER DIVALENT IONS. M. Kavaliers and K.-P. Ossenkopp (SPON: R. Shivers). Depts. of Zoology and Psychology, University of Western Ontario, London, Ontario, Canada N6A 5B7.

It is increasingly apparent that magnetic fields can influence biological systems. Of particular interest is the extremely low frequency band in the range of 50-60 HZ, this being the frequency of alternating currents used as sources of electrical energy by man. One of the more consistent and dramatic effects of exposure to magnetic fields is the attenuation of morphine-induced analgesia. Calcium ($Ca^{++}$) and other divalent ions are considered to be involved in the control of the actions of opiates, while it has been hypothesized that magnetic fields alter neuronal $Ca^{++}$ binding. We report here that acute (1 h) exposure to 0.5 Hz magnetic fields reduces morphine-induced analgesia in a manner consistent with effects on $Ca^{++}$ ions.

In experiment 1 the effects of $Ca^{++}$ on morphine-induced analgesia in male CF-1 mice was examined. Intracereboventricular (i.c.v.) injections of low doses of $Ca^{++}$ antagonized morphine sulfate (10 mg/kg)-induced analgesia, while $Ba^{++}$ was without effect. The chelator, EGTA, given i.c.v., blocked this inhibitory effect of $Ca^{++}$, while the ionophore, A21387, potentiated the antagonistic effects of $Ca^{++}$. EGTA, A21387, or saline, given i.c.v., had no effects on morphine-induced analgesia. These results confirm previous findings of $Ca^{++}$ involvement in the actions of opiates. In experiment 2 exposure of mice for 1 h to a 0.5 Hz rotating magnetic field (RF, 3-90 gauss) reduced morphine-induced analgesia. This inhibitory effect of the RF was blocked by the chelator, EGTA, and augmented by i.c.v. administration of the ionophore A21387.

The results of these experiments show that exposure to magnetic fields alters morphine-induced analgesia in mice in a manner compatible and consistent, with effects on $Ca^{++}$ (and possibly other divalent ions) that are involved in the actions of opiates.

374.7 DIFFERENTIAL HOUSING IN MICE: EFFECTS ON BEHAVIORAL RESPONSES TO OPIATE AGONISTS, ANTAGONISTS AND ETHANOL. G.C. Teskey, R. Book, and M. Kavaliers. Department of Zoology, University of Western Ontario, London, Ontario, N6A 5B7.

Social housing conditions (grouped/isolated) have been shown to affect the behavioral and physiological responses of many species of animals. These socially related behavioral modifications have been attributed to changes in monoaminergic activity. Endogenous opioid systems, which have a major role in the mediation of emotional and stress responses, have also been proposed to be affected by, and be involved in, the determination of behavioral responses to social housing conditions. We describe here the effects of short (1 week) and longer term differential housing conditions on the nociceptive responses of mice to various opiate agonists, antagonists, and doses of ethanol.

Male CF-1 mice of two size classes (20-25 or 30-40 gms) were housed either singly or in groups of 5 for designated time periods. Mice were peripherally injected with either the mu opiate agonists levorphanol, the kappa opiate agonist U-50,488, the sigma opiate agonist SKF-10,047, the mu opiate antagonist naloxone, the delta opiate antagonist ICI-154,129, saline (control) or ethanol (0.5, 1.0, 2.0 g/Kg). Nociceptive responses of the mice, as determined by the hot-plate test, were measured 30 minutes following injection.

There were significant differences in response latencies between the grouped and singly housed mice to various opiate agonists, antagonists and ethanol. Differences were evident after both short (1 week) and longer differential housing. These differences in responses may be attributed to changes in both specific opioid systems and dopaminergic and other monoaminergic neurotransmitters.

374.8 DECREASED SENSITIVITY OF ISOLATION-REARED RATS TO MORPHINE AS MEASURED IN THE CONDITIONED TASTE AVERSION PARADIGM. S. Schenk*, T. Hunt*, G. Klukowski* and Z. Amit (SPON: Z.W. Brown). Center for Studies in Behavioral Neurobiology, Concordia University, 1455 de Maisonneuve Blvd. W., Montreal, Quebec H3G 1M8.

Isolation rearing has been shown to alter the sensitivity of mature rats to dependence-inducing drugs. In the present study, we compared the sensitivity of differentially reared rats to various doses of morphine in a conditioned taste aversion (CTA) paradigm.

Rats were obtained immediately post-weaning (21 days) and housed either singly or in groups of 4 per cage for 6 weeks. They were then placed on a deprivation schedule, allowing them access to fluids for a 20 min. period per day. Following 7 days habituation to this schedule, the CTA experiment began. The deprivation schedule was maintained throughout the 30 day experiment.

Every 6th day, the rats were presented with a solution of 0.1% Na Saccharin for 20 min. Immediately following the presentation, they were given an injection of morphine (0, 2.5, 5.0, 10.0 or 20.0 mg/kg, i.p.). Saccharin consumption was recorded. In all, 5 such pairings of saccharin and morphine were made. In the intervening days, the rats were presented with water for the daily 20 min. period.

The rats that received saline injections following the presentation of the novel tasting solution steadily increased their consumption of saccharin over the 5 pairing days. For both housing conditions, the rats that received a dose of 2.5 mg/kg failed to show such an increase (prolonged neophobia) although they also failed to decrease their consumption of saccharin across pairings. ANOVAs were performed on the change in saccharin consumption from the first pairing day to subsequent pairings for each dosage group. There was a significant interaction between the early housing environment and the pairing days for the rats that received 5.0 mg/kg and 10.0 mg/kg. The group housed rats showed a larger magnitude of CTA than did the isolation housed rats. These findings support the notion that drug sensitivity in the adult can be influenced by environmental conditions.

374.9 DIFFERENTIAL EFFECTS OF GONADAL HORMONES ON MORPHINE-INDUCED ANALGESIA IN MALE AND FEMALE MICE. J. F. DeBold and K. A. Miczek. Department of Psychology, Tufts University, Medford, MA 02155.

In a pilot study we observed that pregnant and lactating female mice were less sensitive than males to the analgesic properties of morphine. The present experiment investigated this apparent sex difference by manipulating testosterone and estradiol levels in both male and female mice and then assessing the animals' response to a wide range of morphine doses.

Adult male and female mice were gonadectomized and then 2 weeks later implanted s.c. with a silastic capsule. The silastic capsules contained one of 6 hormone treatments: 100% testosterone in a 7.5 mm capsule, 10% testosterone (7.5 mm), cholesterol (7.5 mm), 100% estradiol (2.5 mm), 10% estradiol (2.5 mm), or cholesterol (2.5 mm). There were 8 males and 8 females in each hormone group. Testing for morphine response began 1 week after hormone implantation. Baseline levels of the tail flick response and rectal temperature were established in each animal 15 minutes prior to an i.p. injection of morphine (0, 3.0, 6.0, 10.0, 17.0, or 30.0 g/kg morphine sulphate in saline). Animal temperature and tail flick response were again recorded at 15, 30, 60, 90, 120 and 180 minutes after injection.

Estradiol treatment inhibited morphine-induced analgesia in ovariectomized females but not in castrated males. 10 mg/kg morphine induced analgesia in ovariectomized cholesterol implanted mice, but 17 mg/kg morphine was required in estradiol implanted females. In addition, at each morphine dose the estradiol females were less analgesic than cholesterol females. The testosterone capsules had relatively little effect on the response to morphine in ovariectomized females. Males, however, showed a very different steroid-morphine interaction. Castrated male mice with cholesterol implants were somewhat less sensitive to morphine than the testosterone or estradiol implanted castrated males. Testosterone and estradiol treated males were significantly analgesic with 10 mg/kg morphine but 17 mg/kg morphine was required in the cholesterol treated castrated males.

These results demonstrate that sex differences in morphine-induced analgesia can be altered by gonadal steroids, but that males and females are differentially responsive to the effects of estradiol and testosterone on morphine sensitivity. This difference in response to estradiol and testosterone may be the result of hormone effects during sexual differentiation.

374.10 **EFFECTS OF ETHYLKETOCYCLAZOCINE ALONE AND IN COMBINATION WITH TRIPELENNAMINE ON REWARDING BRAIN STIMULATION.** **E.M. Unterwald and C. Kornetsky. Laboratory of Behavioral Pharmacology, Boston University School of Medicine, Boston, MA 02118**

**An increased sensitivity to rewarding brain stimulation has been used as an animal model of drug-induced euphoria. Previous studies have shown that the opiates, morphine and heroin, and to a lesser extent the mixed agonist/antagonist opioids, pentazocine and nalbuphine, lower the threshold for brain-stimulation reward. The euphoria produced by opiates is believed to be mediated by mu opiate receptors. In addition, tripelennamine, an antihistamine, significantly potentiates the modest lowering effect produced by pentazocine or nalbuphine. The present study evaluates the effects of ethylketocyclazocine (EKC), a putative kappa opiate receptor agonist, alone and in combination with tripelennamine on the threshold for rewarding intracranial stimulation. Bipolar electrodes were stereotaxically implanted into the medial forebrain bundle - lateral hypothalamic area of male CDF rats. Thresholds for rewarding brain stimulation to this site were determined by using a modification of the psychophysical method of limits. EKC (.004-.125 mg/kg) administered subcutaneously failed to produce a significant change in reward threshold in any animal. Higher doses incapacitated the animals to the extent that they could no longer perform the task. A dose of 2.5 mg/kg of tripelennamine, which did not cause a significant change in reward threshold, was then administered concomitantly with the various doses of EKC. These combinations produced a dose-dependent lowering of reward threshold which was not seen when EKC was administered alone. The results of this study support the contention that EKC not only has kappa agonist properties but also has some activity at mu receptors. This activity appears to be low but is significantly potentiated by the addition of tripelennamine.**

**(Supported in part by NIDA grant DA02326 and by NIDA Research Scientist Award (CK) K05 DA00099).**

374.11 LOW INTENSITY 60-HZ MAGNETIC FIELDS AND EPILEPSY: REDUCED INCIDENCE OF LETHAL PENTYLENETETRAZOL INDUCED SEIZURES IN RATS PREEXPOSED TO MAGNETIC FIELDS. K.-P. Ossenkopp, D.P. Cain and S.M. Smith. Dept. of Psychology, Univ. Western Ontario, London, Ontario, Canada N6A 5C2.

A growing body of evidence indicates that magnetic fields can influence a number of biological processes. Of special concern is the possibility that natural and man made sources of low intensity magnetic fields, especially those in the 50 to 60-Hz frequency range, can affect the electrical activity of the brain. For example, a significant correlation between seizures in humans and geomagnetic field changes has been noted (Neurosci. Lett., 24:187-191, 1981). In the present series of experiments we examined the effect of brief (1 hr) pre-exposure to 60-Hz low intensity (1 gauss) magnetic fields on pentylenetetrazol (PTZ) induced seizures in male hooded rats. The magnetic fields were produced by two large (100 cm diameter) Helmholtz coils. In Experiment 1 the rats were pre-exposed to the 60-Hz field or to a sham exposure condition (the coils were not energized) and then given an intraperitoneal (i.p.) injection of 55 mg/kg PTZ. Pre-exposure to the magnetic field resulted in a significant reduction in the frequency of lethal seizures ($p < .05$) and in significantly shorter total durations of seizures ($p < .02$). Experiment 2 examined the effects of pre-exposure to the magnetic fields on seizures induced by different doses of PTZ. Animals were pre-exposed to the field or to sham conditions and then injected i.p. with 45, 60, or 75 mg/kg PTZ. Analysis of the data revealed a significant drug dose effect on mortality and on seizure duration ($ps < .01$), but only a nonsignificant trend for reduced mortality in the magnetic field pre-exposed rats at all drug dose levels. In the last experiment rats were again pre-exposed to the magnetic field or sham exposure condition and then injected with 60 mg/kg PTZ. Data analysis indicated a significant reduction in mortality for the magnetic field exposed animals ($p < .05$) and a nonsignificant trend for shorter total seizure duration. When data for those rats injected with 55 or 60 mg/kg PTZ from all three experiments were combined, a highly significant reduction in mortality ($p = .001$) was apparent in those animals pre-exposed to the 60-Hz magnetic field (Magnetic field = 8.3% mortality; Sham exposed = 36.2% mortality). Clearly, the experimental manipulation protected against the lethal effects of PTZ induced seizures.

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374.12 MAGNETIC RESONANCE IMAGING AND MORPHINE-INDUCED ANALGESIA: SOME TEMPORAL EFFECTS OF THE TIME VARYING MAGNETIC FIELD. F.S. Prato*, K.-P. Ossenkopp, M. Kavaliers and E. Sestini* (SPON: T.E. Feasby). Research Institute, St. Joseph's Hospital and Depts. Psychology and Zoology, University of Western Ontario, London, Ontario, Canada N6A 5C2.

A large body of research has shown that magnetic fields can influence a number of biological processes. Of special interest are the recent demonstrations that the magnetic and radio-frequency fields associated with Magnetic Resonance Imaging (MRI) can alter murine morphine-induced analgesia. Both day- and nighttime exposure results in strong inhibition of the analgesic effect with stronger inhibition at night. In the present experiments we exposed adult male CF-1 mice to the time varying magnetic field (TVMF) component of the MRI procedure during the mid-dark period since this component was shown previously to produce the greatest inhibitory effect. The mice were injected intraperitoneally with 10 mg/kg morphine sulfate and tested 30 min later for nociceptive reaction to a thermal stimulus (hot plate) to assess analgesia levels. In Experiment 1 exposure of the mice to the TVMF for 4.86, 11.6 and 22.5 min prior to drug injection (during the mid-dark phase) resulted in increasing inhibition of the morphine-induced analgesia levels as a function of exposure length ($p < .001$). In Experiment 2 mice were exposed to the TVMF for 45 min and tested for morphine-induced analgesia either immediately or 24 hr later. The mice tested immediately showed complete inhibition of the analgesic effect ($p < .01$), whereas the mice tested 24 hr later had normal levels of analgesia. Thus, the effects of the TVMF had dissipated by 24 hr post-exposure. In the last experiment we examined the effects of exposing the mice to the TVMF before or after the morphine injection. Exposure to the TVMF for 22.5 min resulted in an equivalent and significant ($p < .01$) degree of inhibition of the pre- and post-injection exposed animals. These experiments show that the TVMF from MRI can alter the behavioral effects of morphine in a systematic manner and that these effects dissipate within 24 hr.

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374.13 SINGLE-TRIAL CONDITIONED PLACE PREFERENCE USING INTRAVENOUS MORPHINE. M. T. Bardo and J. L. Neisewander.* Dept. of Psychology, University of Kentucky, Lexington, KY 40506.

In three experiments, we assessed the reinforcing effect of acute intravenous morphine. Adult male rats were implanted with a jugular cannula made of Clay Adams polyethylene tubing (PE 50). At 2 or 3 days after surgery, single-trial place conditioning was conducted in a 3-compartment rectangular apparatus with one white and one black end compartment, and a small center gray compartment separated from the end compartments by guillotine doors. Animals were conditioned by pairing morphine with the white compartment for 30 minutes. On an alternate day, animals were exposed to the black compartment for 30 minutes without drug. Following conditioning, animals were given access to all 3 compartments for 15 minutes. Conditioned place preference (CPP) was defined as a significant increase in duration spent in the white compartment relative to saline-injected controls.

In Experiment 1, each animal was injected i.v. with either 0, 4 or 8 mg/kg morphine sulfate immediately after being placed into the white compartment. In Experiment 2, each animal received 2 i.v. injections, one 15 minutes after and one 25 minutes after being placed into the white compartment; the injection sequence was either 8 mg/kg morphine-saline, sal-morph, or sal-sal. In Experiment 3, each animal received 3 i.v. injections, one immediately after, one 15 minutes after, and one 30 minutes after being placed into the white compartment; the injection sequence was either 8 mg/kg morph-sal-sal, morph-sal-2 mg/kg naloxone, morph-nalox-sal, nalox-sal-sal, or sal-sal-sal.

Single trial CPP was dose-dependent when morphine was given immediately after placement into the white compartment (Experiment 1). No CPP was obtained when morphine was delayed 15 or 25 minutes after placement into white (Experiment 2). Naloxone blocked single-trial CPP when given 15 minutes, but not 30 minutes, after morphine, although naloxone did not alter preference when given alone (Experiment 3).

(Supported by USPHS grant DA03487).

374.14 ENDOGENOUS OPIATE INVOLVEMENT IN LEARNED HELPLESSNESS AND ASSOCIATED CHANGES IN BRAIN NOREPINEPHRINE METABOLISM. R. B. Hemingway* and T. G. Reigle. Dept. of Pharmacology & Toxicology, Univ. of Georgia, College of Pharmacy, Athens, GA 30602.

Naloxone (3 mg/kg, i.p.) was administered either 10 min before or 48 hr after exposure of male Sprague-Dawley rats to an uncontrollable footshock stress in order to evaluate the contribution of endogenous opiate systems to the induction and expression of learned helplessness (LH). Escape latencies in a two-way shuttlebox task served to demonstrate the development of LH when compared to latencies obtained with paired animals exposed to escapable footshock and non-shocked controls. The shuttlebox escape deficit (LH) elicited by uncontrollable footshock was prevented by the administration of naloxone prior to the initial stress. However, naloxone given immediately prior to the shuttlebox test, 48 hr after the initial footshock, had no effect on LH. Thus, while endogenous opiate systems appear to be involved in the initial induction of LH, the ultimate expression of this behavioral deficit does not seem to be opiate dependent.

Immediately after shuttlebox testing, rats were sacrificed and the brain was removed and dissected. The brainstem and hippocampus were assayed for two norepinephrine (NE) metabolites, 3,4-dihydroxyphenylethylene glycol (DOPEG) and 3-methoxy-4-hydroxyphenylethylene glycol (MOPEG), by HPLC with electrochemical detection. Since DOPEG is mainly formed by presynaptic monoamine oxidase, while MOPEG can be formed by both pre- and postsynaptic metabolic pathways, the MOPEG/DOPEG ratio was used as a qualitative index of NE activity. In both the hippocampus and brainstem, the MOPEG/DOPEG ratio in animals exhibiting LH was significantly ($p<0.01$) lower than the ratio observed in animals exposed to escapable footshock or non-shocked controls. In animals which received naloxone 48 hr. after uncontrollable footshock (LH not affected), the MOPEG/DOPEG ratio remained significantly ($p<0.01$) decreased. However, when naloxone was given prior to the initial footshock stress (LH prevented), no significant difference in the MOPEG/DOPEG ratio was observed among the three experimental groups. Thus, although LH appears to require an endogenous opiate mechanism for induction, its expression is associated with a decrease in brainstem and hippocampal NE activity. This is in agreement with the biogenic amine hypothesis of depression and serves to enhance the acceptance of LH as an animal model for this disorder.

374.15 DISSOCIATION OF FUNCTIONAL TOLERANCE FROM OTHER TYPES OF TOLERANCE TO THE EFFECTS OF MORPHINE ON SCHEDULE-CONTROLLED BEHAVIOR. R.E. Solomon*, E.A. Wasserman* and G.F. Gebhart. Departments of Pharmacology (College of Medicine) and Psychology, University of Iowa, Iowa City, IA 52242.

Following the original reports by Schuster et al. (J. Exp. Anal. Behav., 4: 327; Psychopharm., 9: 170), there have been numerous demonstrations of tolerance to the behavioral actions of a variety of drugs which is not entirely accounted for by traditional theories of tolerance. The term functional (or behavioral) tolerance has been used to describe mechanisms which, in addition to pharmacokinetic and pharmacodynamic changes, contribute to the development of tolerance. An experiment was designed to determine whether functional mechanisms alone may account for decreases in the initial behavioral effects produced by morphine. Eight rats were trained daily to respond on a two lever, multiple trial, multiple differential-reinforcement-of-low-rate fixed-ratio (mult DRL FR) schedule of food reinforcement. Reliable stimulus control was maintained when sessions were conducted just twice per week. Following saline injections, there was a greater than 7-fold inter-component difference in response rates, but the rates of reinforcement in the two components were not significantly different. The effects of cumulative doses of morphine or chlordiazepoxide were tested once per week; saline injections were given in the intervening sessions. Initially, both morphine (1.0 - 8.0 mg/kg) and chlordiazepoxide (4.0 - 32.0 mg/kg) produced dose-related decreases in response rates and reinforcement rates in both the DRL and FR components. The effects of morphine were similar in the two components; chlordiazepoxide produced greater decreases in FR than in DRL response rates, but similar effects on rates of reinforcement in the two components. The same doses of morphine were then evaluated weekly until it was evident that their initial effects had diminished. As an independent measure of tolerance development via traditional mechanisms, hot plate tests were conducted in the same animals 15 minutes after the initial and final morphine sessions. The selective development of tolerance to the effects of morphine administered once weekly on schedule-controlled behavior, but not, in the same animals, to its antinociceptive effects, would appear to constitute evidence that functional mechanisms alone may be responsible for the development of tolerance to the behavioral effects of morphine. Lack of cross-tolerance to chlordiazepoxide would demonstrate the pharmacological specificity of this effect, and would suggest the interpretation of these data with regard to a state-dependent learning hypothesis or a habituation model of tolerance. Supported by DA 02879 and T32 GM 07337.

374.16 A COMPARISON OF THE RELATIVE POTENCY OF HEROIN AND MORPHINE ON BRAIN-STIMULATION ESCAPE AND REWARD. C.B. Hubner and C. Kornetsky Laboratory of Behavioral Pharmacology, Boston University School of Medicine, Boston, MA 02118.

Previously we reported (Hubner and Kornetsky, Soc. Neurosci. Abst., 10(2):1106, 1984) that heroin (diacetylmorphine) is approximately thirty times more potent than morphine sulfate in lowering the threshold for rewarding brain stimulation. Although reports in the literature suggest that it is only 2-10 times more potent than morphine as an analgesic, we felt it was important to determine the relative analgesic potency of heroin to morphine in our laboratory. In the present study, we compared the analgesic effects of heroin to our previous findings with morphine using a model that makes use of aversive electrical stimulation to the mesencephalic reticular formation (MRF) (Marcus and Kornetsky, Psychopharmacologia, 38:1-13, 1974).

Bipolar stainless steel electrodes aimed at the MRF were stereotaxically implanted in male albino rats (CDF - Charles River Laboratories). Animals were trained to turn a wheel manipulandum to escape from aversive electrical stimulation to this brain site. Determination of the brain-stimulation escape thresholds was accomplished by using a modification of the psychophysical method of limits. The results indicate that heroin, subcutaneously administered, caused a dose-related increase in the threshold, with the minimally effective dose being approximately 0.50 mg/kg. In this paradigm the minimum threshold raising dose of morphine is approximately 5.0 mg/kg. Morphine, unlike heroin, is only slightly more potent in lowering the threshold for rewarding stimulation than in raising the escape threshold. Since the active metabolites of heroin are 6-acetylmorphine and morphine, this increased effect on rewarding brain stimulation suggests a more selective effect of 6-acetylmorphine on those receptors mediating the rewarding rather than the analgesic effects. (Supported in part by NIDA grant DA 02326 and NIDA Research Scientist Award (CK) K05 DA 00099).

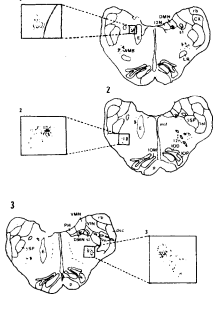
## SLEEP

375.1 CHOLINE ACETYLTRANSFERASE CONTAINING CELLS IN THE BRAINSTEM: RELATIONSHIP TO CARBACHOL MICROINFUSION SITES. P. Shiromani, E. Sutin*, D. A. Armstrong and J. C. Gillin. Department of Psychiatry, UCSD and VA Medical Center, San Diego.

Evidence from brainstem cholinergic infusion studies supports a cholinergic-REM sleep link. Anatomical evidence, however, indicates that in the pontine regions where carbachol readily evokes REM sleep, there are very few muscarinic receptors and even fewer cholinergic cells. In this study we investigated the location of choline acetyltransferase (ChAT) containing cells in relationship to carbachol microinfusion sites.

Two cats were implanted, under Nembutal anesthesia, with sleep recording electrodes and two cannulas in the brainstem. Initially, carbachol (4 ug/0.5 ul Ringers) was infused into each site to determine the REM sleep response. Two weeks after the end of the carbachol test period, one infusion each of GABA (4 ug/0.5 ul Ringers), VIP (100 ng/0.5 ul Ringers) or Ringers (0.5 ul) was made in each site and sleep recordings obtained for at least 6 hours. The order of the infusions were randomized and at least 48 hrs were allowed between infusions.

Compared to Ringers, GABA (n=2) and VIP (n=2) produced a slight decrease in REM sleep (Mean Ringers=20 %; GABA=16%; VIP=14%). The latency to REM sleep was increased considerably (Mean REM latency Ringers=75 mins; GABA=150 mins; VIP=170 min). Carbachol infusions produced either dissociated REM sleep components (see figure legend) or complete REM sleep within 10 mins of infusion (n=2).



Localization of ChAT labeled perikarya (each dot represents one labeled cell) in medulla of one cat. Note relative position of immunolabeled neurons to cannula tract (E). Cholinergic cells are observed in the dorsal motor nucleus of the vagus (insert 1) and nucleus ambiguus (inserts 2 and 3). Carbachol infusion at this site produced increased clusters of PGO waves coupled to nystagmus. This may have occurred as a result of vestibular stimulation. While we did not find any dark staining ChAT neurons in the vestibular nuclei, others have reported high levels of ChAT (Burke and Fahn, Brain Res,328:, 1985) and muscarinic receptors (Wamsley etal, J Neurosci,1:, 1981) in the vestibular complex.

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375.2 REM SLEEP AND MUSCARINIC RECEPTOR BINDING IN RATS ARE AUGMENTED DURING WITHDRAWAL FOLLOWING CHRONIC SCOPOLAMINE TREATMENT. E.L. Sutin*, P. Shiromani, J.R. Kelsoe Jr.*, F.I. Storch*, and J.C. Gillin. University of California, San Diego and VA Medical Center, La Jolla, California, 92161

Much evidence suggests that REM sleep is regulated by pontine muscarinic receptors. In cats, rats, and humans cholinomimetics readily evoke REM sleep. In normal humans, a 3-day chronic treatment in the morning with scopolamine decreases the latency to REM sleep during the night. This suggests that chronic scopolamine treatment upregulates muscarinic receptors which might then influence REM sleep. We sought to determine, in rats, whether during withdrawal from chronic scopolamine there is a REM increase correlated with brainstem muscarinic receptor upregulation.

21 male Sprague-Dawley rats (200-550 gm) were implanted with chronic indwelling EEG and EMG sleep recording electrodes. After a recovery week and two adaptation days, two 6 hour baseline sleep recordings were obtained starting at 9 AM. A randomly assigned experimental group received an injection of scopolamine (10 mg/kg, IP) every morning for 7 consecutive days, while the control group received saline injections only. A 6 hour sleep recording was obtained after the first and last injections and on the subsequent two withdrawal days After the second withdrawal day the animals were decapitated, their brains rapidly removed, and several brain areas were grossly dissected and frozen.

Muscarinic receptor binding was determined by incubating crude membrane preparations of the brain areas (1 mg tissue/ml) with 1 nM 3H-QNB for 90 minutes at 37°C in 2 ml Na-K phosphate buffer. Incubation was terminiated by filtration and 3 quick 5 ml washes. Specific binding was defined as the total binding minus binding in the presence of 0.01 mM atropine.

Preliminary data indicate that scopolamine treated animals showed a significant increase in REM sleep during the first (+29%; $t=5.18$; $df=6$; $p<.05$) and second (+40%; $t=4.56$; $df=6$; $p<.05$) withdrawal days compared to baseline. There were no significant changes in total sleep time or non-REM sleep. No changes in REM sleep were observed in the control group.

Scopolamine produced significant increases in binding in the caudate (+32.0%, $t=5.03$, $df=8$, $p<.01$) and hippocampus (+24.6%, $t=3.24$, $df=16$, $p<.01$). There was no significant change in cortex (+7.3%), brainstem (+1.0%) or cerebellum (+19.8%). Subsequent scatchard analysis revealed an increase in Bmax and not Kd.

These preliminary findings indicate that there is a significant REM augmentation during withdrawal from scopolamine. Contrary to our prediction, there was no increased binding of muscarinic receptors in the brainstem, an area which is implicated in REM generation. Possible explanations for this are discussed.

Research supported by SDVAMC and NIMH-38738

375.3 EXOGENOUS AMINOACIDS, SLEEP SPINDLES AND DREAMS. J. GARCIA-RAMOS Dept. of Physiology, Biophysics and Neurosciences. Centro de Investigación y de Estudios Avanzados del I.P.N. México 14, D.F. MEXICO.

Considering a possible role of aminoacids in the production of dreams, several aminoacids were topically applied over the exposed cortex of barbiturate anesthetized cats. It was found that most of them, putative excitatory or inhibitory, favored the production of sleep spindles although reducing the surface negative waves. Looking for the possible mechanisms involved, observations were made on isolated islands of the same cerebral cortex and on the terrapin turtle cortex. The results showed that all the aminoacids tested depress the surface negative component of the direct cortical responses, the evoked potentials and particularly that of the strychnine spikes. At the same time they facilitate the appearance of oscillatory responses. Since the cortical excitability did not seem to be increased some other change was thought to be implicated. The possibility of a functional opening of low-resistance junctions is suggested by the light increase in the electrical impedance of the tissue after the local application of the aminoacid. The magnitude of the effects were greater for glycine, followed by GABA, alanine, glutamate and aspartate. The present results showed that in order to initiate an oscillatory response it is necessary the presence of a synchronous activation of a relatively large group of dendrites and it is postulated that by electrical coupling another dendritic cluster would be later activated via low resistance junctions and this in turn would activate the first one by the same route to establish a circus movement. The cortical oscillations would trigger the pyramidal neurons which would start the activation of the neural circuits involved in the imagery of dreams.

375.4 SLEEP STRUCTURE AND ITS PARAMETERS IN RELATION TO PARADOXICAL SLEEP DEPRIVATION AND PENICILLIN INDUCED PETIT MAL-LIKE EPILEPSY IN CATS. S. Janković*, R. Veskov*, V. Šušić* and J. Ivanuš*, Inst. for Biological Res., Beograd, 29. Novembra 142, Yugoslavia (SPON: European Neuroscience Association)

Five adult male cats were implanted under Nembutal anesthesia with cortical and subcortical electrodes and prepared for polygraphic recording of sleep. We examined the effects of intramuscular Crystalline Penicillin (P) injections on the parameters of sleep and sleep structure prior to and after the 72 hours of paradoxical sleep (PS) deprivation (SD) as well as the influence of different phases of wakefulness-sleep cycle on P generalized petit mal-like epileptiform discharges.

P prolongs the duration of total sleep time (TST) while SD prolongs it even more. P and SD together render TST shortened. P prolongs the duration of slow wave sleep (SWS) while SD shortens this effect. The number of changes of sleep phases after P administration is significantly higher (leading to a frequently interrupted sleep pattern) as compared to control and postdeprivation sleep. SD antagonize this P effect. SD increases the frequency of PS phases while P, alone or with SD, has no significant effect. In any of these situations mean duration of PS phases is not substantially affected. SD shortens the latency of PS onset while P, either alone or with SD, has no significant effect.

Wakefulness after sleep onset (WASO) is shortened after P but not significantly. SD significantly shortens WASO as compared to control and P. P with SD render WASO prolonged in respect to the effect of SD alone. Thus P, having no effect by itself, partly antagonize the shortening effect of SD. Total number of awakenings (TNAw) is significantly higher after P and lower after SD, as compared to control. After simultaneous application of P and SD, TNAw is significantly decreased in respect to the effects of P. It follows that the SD effect oppose the intensity of P effect.

Since P and SD have the influence on sleep in the same direction when they act separately and when applied at the same time they counteract each other we are postulating that P and SD interaction is antagonistic.

P induced epileptiform discharges are more frequent and characteristically modified during SWS as compared to discharges during awake (Aw) and PS. Correlation of brain epileptiform activity and periferal, muscle, component during SWS approaches zero, while during Aw equals one. During PS the discharges are significantly reduced; when they appear they are much alike the discharges during Aw, but they differ in having no electromyographic correlates.

375.5 PINEALECTOMY INCREASES REM SLEEP DURING THE EARLY LIGHT PHASE OF THE DIURNAL CYCLE OF RATS. J. V. Martin,* R. J. Salin-Pascual,* L. Tamarkin* and W. B. Mendelson* (SPON: P. Skolnick). National Institute of Mental Health, 9000 Rockville Pike, Bethesda, Maryland 20205, USA

In order to clarify the role of the pineal gland in the regulation of sleep, the effects of pinealectomy and injection of melatonin on electroencephalographic (EEG) sleep stages were studied in 300-350 g male rats.

One group of rats was pinealectomized 30 days prior to implantation of cortical and electromyographic electrodes. A second group of rats was simply implanted with the electrodes. All animals were then allowed to recover for two additional weeks before testing. During the first week of testing, each animal was injected either with 250 μg/kg of melatonin or vehicle at 10:00 AM and the EEG was recorded for two hours. A second two hours of EEG was recorded beginning at 2:30 PM that afternoon. In the second week of the experiment, the animals were given the drug treatment not received the first week and the EEG was recorded as before.

No significant effects on sleep latency or duration were observed in this study. However, analysis of variance showed a highly significant effect of pinealectomy ($p<0.0001$) on total time in REM sleep during the two-hour test period.

Minutes of REM Sleep During 2-Hour Recording

| | Pinealectomized | Control |
|---|---|---|
| AM | | |
| Melatonin | 13.0±2.3 | 3.2±1.0 |
| Vehicle | 16.2±2.5 | 2.2±0.6 |
| PM | | |
| Melatonin | 12.8±2.8 | 5.4±0.6 |
| Vehicle | 7.1±1.2 | 7.1±1.5 |

In the morning REM sleep time in pinealectomized rats injected with vehicle was increased approximately seven-fold over that of intact control rats. During the afternoon intact control rats showed a significant increase in amounts of REM sleep and pinealectomized vehicle-injected rats showed a significant reduction in REM sleep as compared to in the morning (Least Significant Difference Post Hoc Tests; $p<0.05$). These findings need not imply that the effect of pinealectomy is simply an advance of the sleep phase, since amounts of REM sleep in the afternoon were the same in vehicle-injected pinealectomized rats and in intact controls. Further, pinealectomized rats injected with melatonin did not show a significant change in REM sleep with time of day. These data appear to implicate the pineal gland as a possible modulator of REM sleep.

375.6 RELATIONSHIP BETWEEN SLEEP PATTERNS AND HYPOTHALAMIC TEMPERATURE CHANGES DURING NORMAL AND BY CHRONIC BARBITURATE TREATMENT. M. Okamoto and V. Adamson. Dept. of Pharmacology, Cornell University Medical College, New York, NY 10021

An abrupt withdrawal from chronic barbiturate treatment often produces insomnia. Duration of insomnia is dependent on intensity of the drug treatment. Hypothalamic thermoregulatory structure has been reported to be intimately involved in the regulation of normal sleep processes. Accordingly, sleep-wake cycles and hypothalamic temperature were continuously monitored in freely moving animals during the control and the barbiturate withdrawal periods through chronically implanted electrodes. Body core temperature was also monitored in order to establish relationship with hypothalamic temperature. During the control period (4 days), 94% of REM sleep incidence was associated with an increase in hypothalamic temperature (Av. +0.207°C) while 69% of SW sleep was associated with a decrease (Av. -0.134°C). Onset of these temperature changes preceded the onset of each comparable sleep stage. Furthermore these hypothalamic temperature fluctuations were less than that of body core temperature. Chronic barbiturate treatment (1 x day, 4 weeks, Na pentobarbital, intragastrically) while the drug was on board, suppressed these temperature fluctuations and also mean hypothalamic temperature. During the withdrawal and during the insomnic period, the fluctuations in temperature were continuously dumped; the degree of rise in hypothalamic temperature was dependent on the intensity of withdrawal. (Supported by the grant DA-00591 from NIDA.)

375.7 THE MORPHOLOGY AND TOPOGRAPHY OF SCALP POTENTIALS RELATED TO PHASIC MEMA IN HUMAN REM SLEEP. M.Fazen*, G.Gerken*, P.Hilton*, D.Slegel*, and H.Roffwarg. Dept. of Psychiatry, Univ. of Texas Health Science Center, Dallas, TX 75235.

We investigated the morphology and scalp distribution of a unique EEG potential that is time-locked to phasic middle ear muscle activity (MEMA) in REM sleep. EEG was recorded at Fz, Cz, Pz, Oz, C5, C6, T3, and T4 in 5 subjects for 3 nights. Bilateral MEMA, EOG, and facial and laryngeal EMG were also recorded. The computer-identified onset of episodic MEMA functioned as a triggering signal ("time-zero") for collection of an EEG epoch about the MEMA trigger ( $\pm$ 750 msec.). Following data acquisition, an averaging program computed the mean, the +/- reference, and a random average for each recording site.

RESULTS: In all 5 subjects, we found an event-related potential (ERP) that begins prior to the initiation of MEMA. It has at least 3 distinct components: a small positive wave, 65-100 msec. before MEMA onset; a prominent negative wave that peaks at or near time-zero; and a large positive component, which reaches its maximum within 260 msec. after MEMA onset. The ERP is stable and synchronous across the 8 recording sites. It is maximal at Cz, and decreases with increasing distance from the vertex. Additionally, an asymmetric increase in positivity on the right side of the skull was noted in the 250 msec. preceding the ERP. The ERP was not observed in either the +/- reference or the random average, both of which emphasize the character of background EEG activity.

The characteristic atonia of the head and neck muscles in REM sleep, the minuteness of the middle ear muscles, the small size of the temporal lobe area ERP, and the fact that the ERP precedes the initiation of MEMA, render it unlikely that this time-locked potential originates in the middle ear muscles. Nor is it likely that EOG contributes artifact to the ERP, since EEG epochs were averaged together without regard to direction of any concurrent saccades. It is possible that brain activity related to eye movements may influence MEMA-related EEG activity, though the ERP scalp distribution suggests otherwise. Also, when only eye movement-free epochs of EEG were averaged, a similar ERP was observed. Though its generating sources cannot be specifically determined from our data, enough similarities exist between the ERP and the auditory evoked potential to suggest that the ERP is cortical rather than sub-cortical in origin and is probably generated in or near the primary auditory projection cortex. Our data, combined with a growing body of work from animal research on the neural pathways transmitting central phasic discharges in REM sleep, allow continuing consideration that the MEMA-related scalp potential reflects, at least in part, an afferent input to the upper brain.

375.8 INTERFERON ALPHA-2 ENHANCES SLOW-WAVE SLEEP IN RABBITS. J.M. Krueger, J. Walter, D. Davenne, S. Shoham and C.A. Dinarello*. The Chicago Med. Sch., North Chicago, IL 60064 and *Tufts Univ. Sch. of Med., Boston, MA 02111

Subjective feelings of sleepiness often occur during the course of infectious disease. The immune response to infectious agents includes increased synthesis and release of interleukin-1 (IL1) and interferon (IFN); both are endogenous pyrogens (1). Prevously we showed that IL1 induces excess slow-wave sleep (SWS) independently from fever induction (2). IFN induces subjective drowsiness in human patients (3) but its effects on SWS are unknown. We now report that IFN induces excess SWS in rabbits. Rabbits were implanted with EEG electrodes, ventricular guide tubes and brain thermistors (2). IFN was human recombinant and provided by Shering Inc.; it contained less that 50 pgm endotoxin per $10^7$ units IFN. IFN was administered either into a lateral cerebral ventricle (ICV) or by intravenous (IV) injection. EEG was recorded for 6 hr after ICV and 5 hr after IV administration of IFN. ICV infusion of $0.2x10^6$ units of IFN failed to affect duration of SWS or body temperature (Table). Significant increases in duration of SWS and body temperatures were observed following ICV infusion of $1-2x10^6$ IFN units and after IV injection of $15x10^6$ units. IFN-induced excess SWS appeared normal in the sense that sleep remained episodic and abnormal behavior was not observed. Pretreatment of IFN with polymyxin B failed to alter IFN-induced sleep responses thus suggesting that responses were not the result of contaminating endotoxin. It is also unlikely that excess SWS was caused by IFN- induced fever; IL1-induced fever and sleep responses could be separated (2) and other substances can induce fever without increasing sleep (4). That IFN and IL1 can modulate sleep may indicate that sleep plays an important role in recuperative processes whether it is recovery from a day's activity or from disease states.

INTRAVENTRICULAR INFUSION

| dose (units) | N | % SWS | | Temp. (3 hr) | |
|---|---|---|---|---|---|
| | | cont. | expt. | cont. | expt. |
| $0.2x10^6$ | 4 | 43±1 | 47±2 | 39.3±0.2 | 39.6±0.3 |
| $1-2x10^6$ | 8 | 45±3 | 62±3* | 39.3±0.1 | 41.0±0.1* |
| INTRAVENOUS INJECTION | | | | | |
| $15x10^6$ | 6 | 47±2 | 60±3* | 39.3±0.07 | 39.8±0.1* |
| $15x10^6$ + 10 ug polymyxinB | 2 | 48-44 | 60-60 | 39.4-38.7 | 40.7-40.7 |

*significantly greater than control values

REFERENCES: 1) J. Clin. Invest. 74:906, 1984. 2) Am. J. Physiol. 246:R994, 1984. 3) J. Am. Med. Assoc. 247:1718, 1982. 4) J. Expt. Med. 159:68, 1984.

375.9 RELATIVE CONTRIBUTIONS OF RESPIRATORY AND CARDIAC RATE AND VARIABILITY MEASURES TO MACHINE DETERMINATION OF SLEEP STATE IN INFANTS. R.M. Harper, Z. Frostig*, T. Hoppenbrouwers*, R.C. Frysinger. Brain Research Institute and Department of Anatomy, UCLA, Los Angeles, CA 90024.

Determination of sleep state in developing infants typically requires human assessment of EEG, eye movement, and body movement criteria. We have previously described utilization of machine-determined cardiac and respiratory measures that classify sleep states with an agreement with human observers that approximates interobserver repeatability.

We have extended these procedures to incorporate additional features of respiratory and cardiac rate variability. These additional features incorporate assessment of frequency and amplitude characteristics of low-frequency variation in heart rate, together with determination of higher-frequency, respiratory-related variation in cardiac R-R intervals.

Twenty-five neurologically normal infants were polygraphically recorded for a 12-hour period (1900-0700 hours) at 1 week and at 1, 2, 3, 4, and 6 months of age. EEG, eye movement, facial muscle, body movement, respiratory, and ECG measures were obtained, and minute-by-minute sleep classifications for all 25 infants were determined from polygraphic tracings by observers trained to a worst-case accuracy of 80% agreement. Twelve infants were used to derive cardiac and respiratory parameters for testing of 13 remaining infants using discriminant analysis (BMDP7M). The minute-by-minute measures included heart and respiratory rate and variability, together with determination of amplitude of respiratory-related variation of cardiac R-R intervals, and amplitude and frequency of long-term variation in R-R intervals. State classifications were made into waking (AW), quiet sleep (QS), and active sleep (REM) only.

A subset of parameters was found useful for state classification across all ages. Additional parameters were useful for classifying state at particular ages. The hierarchy of parameters useful for classification was respiratory variability, followed by heart rate, heart rate variability, respiratory rate, amplitude of respiratory-related variation of R-R intervals, and amplitude of low-frequency R-R intervals. The order of most frequently correctly classified was QS, REM, and AW.

We conclude that machine assessment of periodic motor activity, such as respiration, combined with assessment of autonomic measures, such as heart rate variability, can provide adequate indicators for state classification in normal infants. Variability in these parameters plays a major role in machine decision-making.

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375.10 THE DECREASE OF REM SLEEP BY ATROPINE IS REVERSED BY AUDITORY STIMULATION. G. Arankowsky, R. Aguilar-Roblero*, O. Prospéro* and R. Drucker-Colín. (Spon: Rocío Salceda) Departamento de Fisiología, Centro de Investigaciones en Fisiología Celular, UNAM, México 04510, D.F.

In previous work (Drucker-Colín et. al., Brain Res. 278:308-312, 1983), it was shown that an auditory stimulus given before the appearance of Rapid Eye Movement sleep (REM) increases the duration of this period. On the other hand some pilot experiments suggested that Atropine decreases REM sleep duration.

The present study attempts to determine whether the auditory stimulus is capable of protecting the animal from the effects of atropine on REM sleep.

A group of cats were implanted under pentobarbital anesthesia with electrodes for conventional polysomnographic recording. The cats were divided in four groups, receiving different treatment: Group 1 received 0.2 mg/kg of Atropine I.M., Group 2 was acoustically stimulated during the REM period. Group 3 received both treatments. Group 4 was used as a control.

The sleep-waking cycle of the animals was recorded during an eight hour period. When appropriate according to group 2, a 2 kHz 90 dB auditory stimulus in the form of a beep of 20 ms duration was applied every 20 ms just prior to a REM sleep period and throughout its duration.

The results of these experiments showed that atropine reduced the duration of REM sleep from 7.0 $\pm$ 3.3 to 4.3 $\pm$ 2.2 ($p<.05$). On the other hand the auditory stimulus protected the animals from the effects of atropine, since the mean duration of REM sleep in atropine pre-treated animals given the auditory stimulus was 7.3 $\pm$ 3.8, as compared to 7.0 $\pm$ 3.3 for untreated controls. In addition, the auditory stimulation (Group 2) had a mean duration of 10.0 $\pm$ 3.4, which was significantly different ($p<.01$) from Group 4 duration which was 7.0 $\pm$ 3.3, thus confirming the previously reported increase of REM due to acoustic stimulation. Since both atropine and auditory stimulation have been suggested to impinge upon PGO spike density it is possible that the changes in REM duration are the result of changes in PGO density.

375.11 A COMPARISON OF TWENTY-FOUR HOUR SLEEP RECORDINGS IN GENETICALLY NARCOLEPTIC AND CONTROL DOGS. K.I. Kaitin, T.S. Kilduff and W.C. Dement. Sleep Research Center, Stanford Univ. Medical Center, Stanford, CA 94305.

Two previous studies (1,2) done in this laboratory reported no significant differences in total amounts of wakefulness (W) and slow-wave sleep (SWS) between small-breed narcoleptic dogs and control dogs. It had been our impression from behavioral observation, however, that large-breed, genetically narcoleptic dogs in our colony appeared excessively sleepy. Because excessive daytime sleepiness is a consistent and debilitating symptom of human narcolepsy, we examined whether genetically narcoleptic dogs in fact show evidence of excessive sleepiness.

Three genetically narcoleptic and three control dogs from each of two affected breeds (Labrador retrievers and Doberman pinschers) whelped and reared in our animal breeding facility, were surgically implanted at seven weeks of age with electrodes for recording standard sleep parameters. Dogs were adapted to a 12 hour light/dark cycle and to the recording chamber prior to the first recording which occurred two weeks after surgery. Two continuous 24 hour recordings were made from each animal one week apart. Polygraph records were scored in 30-sec epochs for W, drowsy (D), light sleep (S1), deep SWS (S2), REM sleep (R), and cataplexy (C). Statistical comparisons between narcoleptics and controls of the same breed were made with a two-tailed t-test.

Genetically narcoleptic dogs showed significantly less W than controls of the same breed (27% vs 41% and 26% vs 32% for Labradors and Dobermans, respectively). Moreover, there was an increase in D in the narcoleptic dogs that was statistically significant in the Labrador group (7% vs. 5%). Narcoleptic dogs displayed greater amounts of S2 than controls (18% vs 14% and 20% vs 17% for Labradors and Dobermans, respectively), but S1 was less in the narcoleptic Dobermans (25%) than controls (28%). Although amounts of R were generally less in the narcoleptic dogs, R and C together were significantly greater than R alone in the controls (26% vs 15% and 23% vs 17% for Labradors and Dobermans, respectively).

The results indicate that, whereas genetically narcoleptic dogs show less W than controls, they are not hypersomnolent. However, the high levels of D in the affected Labradors suggest that they are "sleepier" than controls and may be a useful animal model for studying excessive sleepiness.

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2. Mitler M, Dement W. Electroenceph clin Neurophysiol 43: 691-699, 1977.

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375.12 NEUROLEPTIC WITHDRAWAL INDUCED CHANGES IN SLEEP EEG, PROLACTIN AND OTHER CLINICAL PARAMETERS, G. Thaker*, A. Wagman, C. Tamminga, Maryland Psychiatric Research Center, University of Maryland, Baltimore, Maryland 21228

Chronic neuroleptic treatment induces a variety of neuronal changes in brain dopamine (DA) systems which manifest themselves clinically as withdrawal or tardive phenomenona. In order to evaluate the effect of chronic neuroleptic treatment on central dopaminergic systems, the biologic sequelae of antipsychotic drug withdrawal are being studied. Preliminary results are reported here. Seven schizophrenic inpatients, (four with tardive dyskinesia, three without) each treated for at least 4 weeks with haloperidol (40 mg/day) or RO221319, a $D_2$ antagonist (120 mg/day) participated in the study. Four patients also received benztropin (up to 4 mg/ day) for parkinsonian side-effects. Sleep studies were initiated while the patients were on neuroleptics and were continued for seven more days following abrupt drug withdrawal. No medication changes were made during the final week of treatment; in patients already receiving benztropin, it was continued through the seven neuroleptic withdrawal nights. Subsequently, patients were kept drug free for at least four weeks and another set of sleep EEG recordings were obtained. In some patients, twice weekly psychosis and tardive dyskinesia (TD) ratings were obtained; also, hormonal (prolactin and growth hormone) measurements were carried out during chronic antipsychotic drug treatment, first week of withdrawal and at the end of 4-week drug-free period.

Results: A consistent REM suppression was observed in all patients during the first week of drug withdrawal. This effect was short lasting (2-4 days) and occurred on different withdrawal nights in each patient. A peak withdrawal night was determined in each patient when there was maximum change in REM sleep; the mean of 3 consecutive nights, including the night of peak withdrawal effect and nights preceding and following was used in calculations. REM sleep in minutes was significantly decreased during peak withdrawal period (46 $\pm$ 22 min.) when compared to both chronic treatment (85 $\pm$ 17 min.) and 4-week drug-free (79 $\pm$ 15 minutes) periods (Freidman's test, =11.1, df=2, p=0.0038 and post hoc Wilcoxin signed rank test p 0.02) Patients with tardive dyskinesia had a more marked response to neuroleptic withdrawal in that their mean REM reduction was 59 $\pm$ 21% compared to 34 $\pm$ 15% in non-TD patients. In the whole group total sleep and NREM time remained unchanged. Neuroleptic withdrawal effect on other clinical and hormonal parameters will also be discussed. We propose that sleep EEG may provide a sensitive measure of post-neuroleptic DA-receptor hypersensitivity in man.

375.13 EFFECT OF A SEROTONIN AGONIST AND A SEROTONIN REUPTAKE INHIBITOR ON SLEEP IN NORMAL AND REM-DEPRIVED RATS. Ross H. Pastel and John D. Fernstrom. Department of Psychiatry and the Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15213.

Serotonin neurons are thought to be involved in the mechanisms which control REM sleep. Previous experiments in our laboratory have shown that fluoxetine, a selective serotonin reuptake inhibitor, has a selective suppressive effect on REM sleep in rats while having no significant effect on non-REM (NREM) sleep. The selective REM effect is seen in the dose range 0.94-2.5 mg/kg. At higher doses, fluoxetine also increases sleep latency. In the present set of experiments, we compared the effects of fluoxetine with those of trifluoromethylphenylpiperazine (TFMPP). TFMPP is a selective serotonin agonist which does not induce the serotonin motor syndrome [Lucki I, Frazer A. Soc Neurosci Abstr 8:101, 1982]. To test their potency in suppressing REM, each drug was studied under two conditions: administration at the normal onset of the daily light period, and after a three hour period of REM deprivation. Rats were REM deprived by tapping on their cages whenever the EEG showed signs of REM sleep. Each rat served as his own control, receiving i.p. saline and either fluoxetine (2.5 mg/kg) or TFMPP (2.5 mg/kg).

Both drugs had strong REM suppressive effects. TFMPP suppressed REM for over 4 hours when administered at lights on or after REM deprivation. When given at lights on, fluoxetine suppressed REM sleep for three hours. The REM suppressive effect was shorter when fluoxetine was administered after REM deprivation, the effect lasting approximately two hours. In comparing the REM suppressive effects of TFMPP versus the effects of fluoxetine, TFMPP had a longer time course of action and a more complete suppression of REM. Neither drug induced an abnormal EEG at any time. NREM sleep was not significantly different after injection of either drug at lights on. When administered after REM deprivation, fluoxetine again had no effect on NREM sleep, while TFMPP increased wakefulness in the first hour after injection.

Fluoxetine and TFMPP each enhance serotonin transmission, although by different mechanisms, and each drug suppresses REM. This fact suggests that the effects seen on REM are due to the action of these drugs on serotonin neurons. Since fluoxetine was less effective when administered after REM deprivation than at light onset, the findings also suggest that serotonin neurons may be less active when a strong REM pressure is present. The results thus provide additional support for the hypothesis that serotonin neurons are involved in the mechanisms of REM sleep.

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375.14 CHARACTERIZATION OF CARDIAC BEAT-TO-BEAT PERIODICITIES WITHIN SLEEP-WAKING STATES UTILIZING TIME DOMAIN PROCEDURES. Z. Frostig*, R.D. Frostig, and R.M. Harper (SPON: H. Lesse). Brain Research Institute and Department of Anatomy, UCLA, Los Angeles, CA 90024.

Plots of successive cardiac beat-to-beat intervals against time demonstrate at least two prominent periodicities: a high-frequency variation with a period related to respiratory activity (sinus arrhythmia), and low-frequency variation with a period of 10 seconds or more. Amplitude and frequency characteristics of both high- and low-frequency variation provide useful indices of hemodynamic and respiratory action, and may be useful in sleep-state assessment, particularly in infants.

The classic manner of describing these variability changes has been spectral analysis calculated after Fourier transforms. However, frequency domain procedures that average over relatively long time periods are extremely sensitive to occasional artifacts, such as missed beats or very short intervals resulting from double triggering of the cardiac R wave. This study utilized a robust analytical method to characterize periodic variation in R-R data.

"Peaks" and "troughs" in the R-R interval plots are noted when successive R-R interval differences become negative or positive. These points describe high-frequency aspects of the R-R curve. Values for low-frequency variations are derivatives of changes in the high-frequency values. Thus, low-frequency peaks are defined as peaks of the curve formed by high-frequency peaks, and its troughs as the troughs formed by the curve of high-frequency troughs. Relative rise times are used to discriminate aberrant points, which result from artifacts. Median values, being a robust measure relative to the mean, are used to characterize periodicity within each minute, and the following parameters are typically measured: amplitude, period in time, and period in number of intervals for high-frequency changes, and corresponding characteristics of the low-frequency periodicity.

The procedure has been successfully applied to studies quantifying high- and low-frequency variation in heart rate over the first 6 months of life (Schechtman et al., Soc. Neurosci. Abs., 1985) and for infant sleep-state classification using cardiac rate variation as state classification parameters (Harper et al., Soc. Neurosci. Abs., 1985). ANOVA assessment on cardiac rate variation, for example, demonstrates that all parameters listed above can be used to discriminate infant sleep-waking states. Relative to spectral procedures, a much larger data sample is available for analysis using this procedure, since errors introduced by artifacts are very much reduced.

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375.15 THE EFFECTS OF PARADOXICAL SLEEP DEPRIVATION ON EEG POWER DENSITY IN THE RABBIT. R.T. Pivik, F.W. Bylsma* and P. Cooper*. Lab. of Neurophysiology, University of Ottawa, Ottawa, Ontario K1H 8L6

It has been suggested that the pervasive increase in low frequency EEG power density across all sleep stages following total sleep deprivation in man reflects the presence of a unitary sleep-intensity process (Borbely et al., Electroencephalogr. Clin. Neurophysiol., 5:483, 1981). The present investigation was conducted to determine if similar effects would be present following selective deprivation of paradoxical sleep (PS) in the rabbit.

Standard EEG (postcentral cortex), EOG and nuchal EMG activity were continuously monitored for 5 consecutive days (2 days baseline, 1 day PS deprivation, 2 days recovery) in adult New Zealand White male rabbits (n=5). Animals were maintained on 12 hour light-dark cycles with food and water continuously available. PS deprivation was effected by auditory stimuli (900-1000 Hz, 84-87 db tone bursts) delivered at PS onset via speakers mounted in the recording chamber. One minute tape-recorded samples of postcentral EEG activity were digitized (200 samples/sec) and power density was determined for the following frequency bands: 0-2, 2.1-8, 8.1-12, 12.1-15, 15.1-20 and 20.1-50 Hz during wakefulness (W), drowsy (D), slow wave (SW) sleep, and PS. Data were statistically analyzed using analysis of variance procedures with post hoc tests when appropriate.

PS time was significantly reduced during deprivation ($p<.01$) and significantly enhanced (rebound, $p<.05$) during recovery relative to baseline values. Neither total sleep time nor total amount of slow wave sleep was significantly affected by the deprivation procedure.

Post-deprivation recordings did not differ from baseline values in total power. Furthermore, no frequency band showed consistent power density changes either across sleep-wakefulness states or across sleep stages only. However, significant post-deprivation power variations did occur within conditions and included: increased high frequency beta (20.1-50 Hz) during W, $p<.07$; decreased beta (15.1-20 Hz) during D, $p<.02$; increased theta (2.1-8 Hz) and sigma (8.1-12 Hz) during PS, $p<.01$ and $p<.09$, respectively; and, decreased high frequency beta ($p<.02$) during PS.

The absence of significant increases in power density in delta activity (0-2 Hz) and the concentration of effects in PS following PS deprivation (a) suggest that selective sleep deprivation has relatively selective state EEG power density effects; and, (b) do not support the notion of a unitary sleep process indexed by low frequency activity.

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## DEVELOPMENT AND PLASTICITY: MOTOR SYSTEMS

376.1 THE DEVELOPMENT OF RUBROSPINAL PROJECTIONS IN THE NORTH AMERICAN OPOSSUM. T. Cabana* and G.F. Martin (SPON: R.H. Ho). Dept. of Anatomy, The Ohio State University, Columbus, Ohio 43210.

In experiments using retrograde tracing techniques, we defined a developmental sequence as to the origin of descending projections to the spinal cord of the opossum (Cabana and Martin, Devel. Brain Res., 15 (1984) 247-263). Axons from the vestibular nuclei, reticular formation raphe and coeruleus complex reach the cord very early in development; those from other brainstem areas, such as the red nucleus, reach the cord somewhat later, and those from the cerebral cortex reach the cord last. With orthograde tracing, we were then able to describe a sequential innervation of the spinal cord by cortical axons (Cabana and Martin, Soc. Neurosc. Abst., 10 (1984) 321). We are now seeking to describe the intraspinal growth of rubral axons in developing opossums.

Horseradish peroxidase conjugated to wheat germ agglutinin was injected into the red nucleus of 26 pouch young opossums, Didelphis virginiana, and in areas surrounding the red nucleus or in the subarachnoid space of 45 control pups. By postnatal day (PD) 7, a few labeled rubral axons were present in the external part of the contralateral dorsolateral funiculus at all spinal levels. Injections in younger pups labeled rubral axons at the medullo-spinal junction, but not in the cord. We interpret the latter data with caution, however, since injections in the younger animals included only a small rostral portion of the red nucleus. At PD 7, rubral axons did not enter the gray matter except, maybe, at rostral cervical levels. By estimated PD (EPD) 15, the rubrospinal tract was more densely labeled at all spinal levels. Rubral axons arborized in the lateral half of laminae V-VII at upper cervical levels, began to penetrate lamina VI at the cervical and lumbosacral enlargements, but only approached, without entering, the gray matter at thoracic levels. At EPD 17, rubral axons at upper cervical levels were present throughout the lateromedial extent of laminae V-VI, although denser laterally, and in laminae IV and VII. They were restricted mainly to the lateral border of lamina VI at the enlargements and the lateral border of lamina V at thoracic levels. By EPD 25, rubral axons were more densely labeled in laminae IV-VII at all spinal levels and were seen for the first time in lamina III at cervical and lumbosacral levels

These observations suggest that: 1) rubral axons do not innervate the gray matter of a given segment until some time after they have reached that segment, 2) rubral innervation of the spinal gray does not follow an absolute rostral-to-caudal gradient, 3) innervation of each segment occurs in a similar sequence and 4) rubral innervation of the cord is completed prior to cortical innervation of either the cord or the red nucleus (Cabana and Martin, Am. Assoc. Anat. Abst., 1985). Supported by BNS-8309245 and NS-10165.

376.2 MAINTENANCE OF TRANSIENT OCCIPITOSPINAL AXONS IN THE RAT. M.A. Sharkey, R.D. Lund, R.M. Dom*. Department of Anatomy, Medical University of South Carolina, Charleston, SC.

Early in development the pyramidal tract originates from the entire extent of cortical Layer V including the occipital cortex (Stanfield et al., Nature, 298:371-373, 1982). These occipital corticospinal fibers disappear during the first three postnatal weeks, but the cells of origin persist and have been shown to sustain projections to the pons and/or superior colliculus (Stanfield et al., Neurosci., 9:375, 1983).

In this study, embryonic day 15 tectum was transplanted into the pyramidal tract of the cervical spinal cord in newborn Long Evans hooded rats. The tectal transplants were prepared and maintained in a sterile dish containing Ham's F10 culture medium. The newborn rats were anesthetized by cooling with ice. Then superficial layers of the superior colliculi were extirpated, a laminectomy performed on cervical vertebrae 2 and 3, the dura opened longitudinally, and the pia reflected prior to creating a small midline cavity in the dorsal columns. Following this, the fetal tectal tissue was transplanted into this midline cavity, the vertebral bodies reapproximated, and the muscle and fat layers repositioned and sutured. As controls, fourteen newborn rats received superficial tectal ablations without transplants. This control group was allowed to survive for a time period identical to that of the experimental animals that received transplants.

Four to five weeks after transplantation, the animals were anesthetized with tribromoethanol, reopened as above, and the transplant visualized. Using a pipette secured to a Hamilton syringe, horseradish peroxidase, HRP (0.2-0.4 $\mu$l), was injected into either the transplant and surrounding cervical cord or into the corticospinal tract at the C2-C3 level of the spinal cord in the control group; forty-eight hours later the animals were perfused transcardially with phosphate-buffered solutions of 0.5% glutaraldehyde followed by 0.2% glutaraldehyde. The brains and cervical spinal cord were removed, frozen sectioned at 40 $\mu$m, and alternate sections processed with the chromagens diaminobenzidine tetrahydrochloride and tetramethylbenzidine.

HRP labelled cells were demonstrated in visual association areas 18a and 18b at four to five weeks postnatally in the animals receiving tectal transplants. The control animals which received HRP injections into the spinal cord at cervical levels showed no HRP positive cells in the occipital cortex. Thus, the aberrantly placed tectum influences the survival of occipitospinal axons which are normally eliminated during the third week of postnatal life.

376.3 ANALYSIS OF CORTICOSPINAL PLASTICITY USING RETROGRADE FLUORESCENT TRACERS IN RATS. B.S. Reinoso* and A.J. Castro (SPON: T. Khan), Dept. of Anat., Loyola Univ. Stritch Sch. of Med., Maywood, IL 60153.

The rodent corticospinal tract (CST) is predominantly a crossed pathway. However, after unilateral cortical lesions in neonatal rats, a marked increase in ipsilateral CST fibers, originating from the unablated cortex, has been reported. The present study was undertaken to determine whether these fibers represent axonal collaterals of normal crossed projections or whether they arise from a separate population of cortical neurons.

Large lesions of the right cerebral hemisphere of 1-2 day old Long Evans rats (n=9) were made by aspiration. Control animals (n=4) did not receive lesions. At 2-8 months and using sodium pentobarbital anesthesia, laminectomy of 1-2 vertebrae at cervical enlargement levels was performed. Spinal columns were secured with a vertebral clamp and five 0.01 ul injections of 2% fast blue and 0.06 ul of 2% diamidino yellow were spaced 1-2 mm apart rostral caudally and 1 mm deep on the left and right (or vice-versa) side of the spinal cord. After 6 days animals were transcardially perfused with 10% buffered formalin. Fifty um horizontal sections through the cervical enlargement and 40 um sagittal sections through the brain were cut frozen, mounted from water and viewed using an epifluorescent Leitz microscope.

Histological analysis indicated that injection sites in 10 animals were restricted to one side of the cord. The presence of only FB or DY labeled neurons within either of the red nuclei further verified the placement of spinal cord injections. In control animals, numerous FB and DY single-labeled neurons were found predominately contralateral to injections. No doubled labeled cells were found in these animals. In experimental animals, numerous FB and DY singled labeled neurons were found within the unablated hemisphere; no double-labeled cells were found.

The absence of double-labeled cells in the unablated cortex of animals that sustained unilateral cortical lesions indicates that anomalous ipsilateral CST fibers are primarily not collaterals of normal crossed CST fibers. Accordingly, the ipsilateral CST fibers described after neonatal cortical lesions may represent (1) normal fibers that failed to decussate, (2) the development of fibers from neurons that normally do not project to the spinal cord or (3) the abnormal persistence of developmentally transient fibers. (Supported by NIH Grant NS 13230.)

376.4 CORTICALLY-EVOKED HINDLIMB MOVEMENTS IN RATS THAT SUSTAINED NEONATAL SPINAL CORD LESIONS. M.F. Dauzvardis*, G. Kartje-Tillotson and A.J. Castro. Dept. of Anat., Loyola Univ. Stritch Sch. of Med., Maywood, IL 60153.

Corticospinal fibers have previously been reported to grow around partial spinal cord lesions made in neonatal animals (Schreyer and Jones, Neurosci. 9:31, 1983; Bregman and Goldberger, Develop. Brain Res. 9:137, 1983). In view of these findings, the present study examined limb movements evoked by intracortical microstimulation in adult animals that sustained partial mid-thoracic spinal cord lesions at birth or at maturity. Using a sharpened no. 11 scalpel blade, lesions intending to transect the dorsal funiculus, and therefore the path of the corticospinal tract, were made in hypothermic-anesthetized 1-2 day old rats (n=4) and adult rats (n=6) anesthetized with sodium pentobarbital (40mg/kg). Animals without neonatal (n=4) or adult (n=4) lesions served as controls.

Thirty days postoperatively and using Ketamine anesthesia (100mg/kg), intracortical microstimulation (0.25 msec pulses; 350 hz, 300 msec trains, at 5-100 uamps) was applied at a depth of 1.7mm in the forelimb (FL) and hindlimb (HL) motor areas of the right cortex of each amimal. Mean threshold values (i.e., the minimal current needed to evoke movements) for contralateral FL and HL movements in control animals equaled 12 and 25 uamps, respectively. Mean threshold values for FL movements in animals that sustained adult or neonatal spinal cord lesions approximated control values whereas HL movements could not be evoked at current intensities under 100uamps in the adult lesion group. In contrast, evoked HL movements at mean threshold values of 52uamps were observed in the neonatal lesion group. Histological analysis (50um frozen sections, cresyl violet) demonstrated comparable lesions in both experimental groups.

The evoked HL movements observed in mature animals that sustained neonatal spinal cord lesions suggest that the previously described rerouting of corticospinal fibers around neonatal spinal cord lesions may be functional (Supported by NIH Grant NS 13230.)

376.5 DEVELOPMENT OF HINDLIMB STEPPING BEHAVIORS: NEUROMUSCULAR PATTERNS IN NORMAL AND SPINAL NEONATAL KITTENS. N.S. Bradley and J.L. Smith. Dept. Kinesiology, UCLA, CA 90024.

Limited EMG data in neonatal kittens suggest that antagonist hindlimb muscles are not reciprocally activated during stepping prior to onset of weight-supported locomotion at 2-3 wks of age (Scheibel & Scheibel, Exp. Neurol. 29:1970). It has also been suggested that spinalization in the neonate can hasten maturation of hindlimb stepping (Robinson & Goldberger, Neurosci Abst. 8:1982). We examined these notions, using EMG synchronized with video records during airstepping (ASTP), treadmill locomotion, and overground stepping in normal and spinal kittens.

To examine intra- and interlimb coordination during the stepping behaviors, the lateral gastrocnemius (LG), tibialis anterior (TA), and vastus lateralis (VL) were implanted in 12 kittens at 1 day of age. Normal animals (n=4) and those spinalized (T12) at 1 day (n=4) or 14 days of age (n=4) were tested 3 times/wk from 3 to 32 days of age. To elicit stepping motions, each kitten was: held under the chest with hindlimbs pendent (ASTP), held above a treadmill with hindpaws contacting the moving (≤0.2m/s) belt, and encourgaged to step overground while supporting its own weight.

Normals. At 3 days of age, 3-11 consecutive steps with minimal weight support were recorded in all animals on the treadmill with cycle periods (interval between LG burst onsets) of 1067-5169 ms. VL onset (±2% cycle) and duration (60-80% cycle) were tightly coupled with LG. TA onset was variable, often occurring during LG activity and/or LG silence. Contralateral LG onset was synchronous (±10% cycle) or alternating (30-40% cycle). During ASTP, 2-19 steps/bout were recorded at 3-7 days, with similar cycle characteristics. Overground, 1-3 steps/bout were taken, with only one TA burst per cycle (onset 80%, duration 20% cycle). Development of stepping 3-32 days of age was characterized by increases in cycles/bout and decreases in cycle period.

Spinals. Treadmill locomotion and ASTP in spinals 3-32 days old differed from normals: cycle periods were shorter (680-2478 ms), extensor burst durations less (30-50% cycle), and a single, prolonged TA burst began at 30-50% cycle. ASTP persisted the 1st mo in spinals, but seldom occurred in normals after 1-2 wk. During best steps on the treadmill, weight support did not appear to differ between normals and spinals.

Our data show that stepping occurs in the hindlimbs of normal neonatal kittens under several conditions with a tight synergy between extensors, while phasing of flexors is variable, particularly during treadmill locomotion. Shorter cycle periods during stepping behaviors in spinal neonates suggest spinalization removes inhibition by rostral centers, but whether or not it hastens maturation warrants further study. Supported by NS 19864.

376.6 SOMATOTOPIC ORGANIZATION OF IPSILATERALLY PROJECTING CORTICOSPINAL NEURONS IN RATS SUBJECTED NEONATALLY TO ABLATION OF ONE MOTOR CORTEX. W.F. Nation*, M. Cox* and D.R. Humphrey. Lab. of Neurophysiology, Emory Sch. of Med., Atlanta, GA 30322.

If one motor cortex is ablated in the rat on postnatal days (PND) 1-4, the surviving motor cortex develops an 'aberrant', ipsilateral corticospinal projection, in addition to its normal contralateral projection (Hicks and D'Amato, Exp. Neurol., 29: 416). It is not known at present, however, how this ipsilateral system is organized somatotopically within the surviving motor cortex, nor how its development alters the somatotopic organization of the cells origin of the normal contralateral projection. To examine this and related questions, we used the technique of intracortical microstimulation (ICMS) to study the somatotopic organization of the surviving motor cortex, in adult rats subjected on PND 1 to ablation of one motor cortex (N=4); experiments were also performed in normal control animals (N=4). Anterogradely transported HRP was also used to study the course and terminal distributions of normal (N=4) and aberrant (N=4) corticospinal pathways. Finally, pyramidotomies were performed in normal (N=3) and in neonatally ablated adult animals (N=3), to establish that novel motor responses seen ipsilaterally were indeed mediated by fibers traversing the medullary pyramid. Our major findings were as follows.

(1) ICMS (0.2 msec cathodal or biphasic pulses, 100 msec train, 300 pps, 2-120 μA intensities) in the ketamine-sedated (50 mg/Kg) normal animal evoked only contralateral movements, at thresholds ranging from 4-60 μA. Ipsilateral responses could be evoked, but only with macroelectrodes (0.5 mm exposed tip) and at high thresholds (25-150 μA). (2) In the neonatally ablated animal, however, ipsilateral responses were evoked with ICMS at thresholds comparable to those for evoking contralateral movements (4-60 μA). In none of these animals, however, were movements of the digits evoked on either side. (3) The motor cortex map for contralateral responses was basically similar in normal and ablated animals, but the anterior and posterior forelimb zones were less distinctly separated in the latter. (4) For any given joint or body part, the map for ipsilateral responses in the ablated animals was roughly isomorphic with that for contralateral responses, raising the possibility that single neurons may project to similar regions on both sides of the cord. (5) Ipsilaterally terminating corticospinal fibers traveled in both the dorsal and anteromedial columns, with re-decussation occurring at both medullary and spinal levels. (6) Pyramidotomy abolished both ipsi- and contralaterally evoked responses, indicating that the fibers responsible for the former are either corticospinal, or part of a cortico-reticulo-spinal pathway; experiments are currently underway to resolve this point. (Supported by NIH Grant NS 20146).

376.7 CONTRACTILE PROPERTIES OF MOTOR UNITS IN DEVELOPING RAT SOLEUS MUSCLE. H. Lee Sweeney* and Wesley J. Thompson, Depts. of Physical and Health Education and Zoology, University of Texas, Austin, TX 78712.

We are currently investigating the contractile properties of motor units in developing rat soleus muscles. Soleus muscles were dissected in contiguity with the ventral roots L4 and L5 from 8 day and 16-17 day old animals and examined in vitro. Single motor units were isolated by teasing and stimulating ventral root filaments. Previous histochemistry of glycogen depleted units at these ages had suggested that there were two types of units present: those comprised primarily (>70%) of nominally slow fibers and those comprised primarily (>90%) of nominally fast fibers. To ascertain if these two histochemical types of units give rise to two populations of units distinct in their mechanical properties, we measured the twitch kinetics as well as the velocity of unloaded shortening of isolated motor units. At both ages, time to peak tensions were not obviously bimodally distributed. Rather, there seemed to be a continuum of twitch rise times, with the range being greater in the 16-17 day old muscles than in the 8 day muscles. The distribution of the velocities of unloaded shortening is uncertain due to the present small sample size. However, it is clear that the range of unloaded shortening velocities is greater in the older animals. In experiments where the units were first activated separately and then combined, the time to peak tension of the combined units was a weighted mean of the individual units, whereas the unloaded shortening velocity was determined by the speed of the fastest unit.

Thus, the two types of motor units revealed by histochemistry do not clearly translate into a bimodal distribution of the contractile properties of the units. This could be due to factors other than the myosin contained in the fibers. Certainly, in the case of the twitch kinetics, changes in the rate of SR calcium release and uptake could be deterministic. However, in the steady state conditions (i.e. tetanus) during which the velocity of unloaded shortening was measured, it is likely that the velocity differences we see are indicative of contractile protein differences. Thus, it is possible that a broad distribution of myosin isozymes is contained within motor units of developing animals. The conclusions previously drawn from histochemistry could be too simplistic, due to the inability of the ATPase staining to detect subpopulations of fibers within the two broad histochemical classifications. We are continuing to address this issue via simultaneous contractile measurements and glycogen depletion studies.

376.8 NEUROMUSCULAR JUNCTIONS ON EMPTY MUSCLE BASAL LAMINAE IN METAMORPHIC FROG MUSCLE. K. Lynch* (SPON: R. Cowie), Dept. of Anatomy, Uniformed Services University of the Health Sciences, Bethesda, MD 20814-4799.

During and shortly after metamorphosis, a contingent of muscle fibers in the rectus abdominis muscle of Rana pipiens undergoes degeneration (Lynch, K., J. Morphol. 182:317, 1984). Phagocytes fragment the muscle fibers within the basal laminae and phagocytose the fragments. The empty basal laminae persist for days to weeks, compressed between the remaining muscle fibers. We have observed presynaptic elements on such empty basal laminae in a number of muscles from metamorphic and early postmetamorphic animals. Ultrastructurally, these are indistinguishable from the presynaptic elements of normal neuromuscular junctions. The nerve terminals are overlain by Schwann cells and contain the normal assortment of organelles, including synaptic vesicles clustered over active zones. Often there are small, membrane-bounded profiles adherent to the postsynaptic surface of the basal laminae. Some of these have no stainable content; others appear to contain cytoplasm. In the rare instances in which neuromuscular junctions were seen on degenerating muscle fibers, a fragment of muscle which included the postsynaptic specialization was invariably bound to the presynaptic elements by synaptic basal laminae; that is, phagocyte processes did not separate the pre- and postsynaptic elements. Adherent postsynaptic fragments of muscle are thus the likely origin of the small postsynaptic profiles left by phagocytes within otherwise empty basal laminae.

The persistence of structurally normal nerve terminals on empty basal laminae is evidence that the synaptic basal lamina alone is sufficient to induce the axon to maintain its presynaptic specialization. This is consistent with the discoveries of Marshall, Sanes, McMahan and colleagues (reviewed by Sanes, J.R., Ann. Rev. Physiol. 45:581, 1983). The "unilateral" neuromuscular junctions also suggest that denervation plays no role in the selective loss of muscle fibers during metamorphic remodeling.

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376.9 DIFFERENT PROGRAMS OF SYNAPSE DEVELOPMENT IN TWO MUSCLES OF XENOPUS LAEVIS. J.L. Owens and R. Kullberg. Biology Dept., University of Alaska, Anchorage, AK 99508.

A comparative study was made of the development of synaptic currents in the superior oblique and interhyoideus muscles of Xenopus laevis. The superior oblique is an extraocular muscle which produces rapid saccadic movements of the tadpole eye. The interhyoideus is a broad, flat muscle which elevates the floor of the mouth. In order to assay the development of acetylcholinesterase (AChE) activity and acetylcholine receptor (AChR) gating kinetics, we examined the developmental change in miniature endplate current (MEPC) decay constants at endplates in each muscle. The earliest stage at which we were able to detect synaptic activity in these muscles was stage 41 (3 days old). The following developmental changes in MEPC decays were observed. Interhyoideus: MEPC decay constants were initially 5-6 msec. They decreased within 16 hours to 2-3 msec. There was no further change in decay constant until late metamorphosis, approximately 7 weeks later, after which the decay constants underwent a further decrease to about 1 msec. Superior oblique: MEPC decay constants were initially 3-4 msec. They decreased within 11 hours to about 1 msec and remained about that value for the following 4 weeks. They then gradually became longer, reaching values of 1.5-2.0 msec at mid-metamorphosis to adulthood.

Two points of interest emerge from these data: (1) Different muscles within a species may vary markedly in their programs of synapse development, and (2) within a given muscle, there may be more than one distinct phase in the development of synaptic function. The initial rapid decline in MEPC decay in both muscles may be due in part to the development of AChE. Karnovsky stain revealed no localized accumulations of AChE in stage 41 muscle, while such accumulations were apparent 16 hours later in stage 44 muscle. After the development of AChE, subsequent differences in MEPC time course between the two muscles probably represent different relative numbers of slow and fast AChR channels. The longest and briefest MEPC decay constants, recorded in either muscle, were equivalent to the estimated open times of the slow and fast AChR channels known to be present in Xenopus muscle. Some MEPCs in both muscles had biphasic exponential decays, and in such cases the decay constants were comparable to the mean channel open times of the two classes of AChRs. In interhyoideus muscle, the shift to fast MEPC decay constants can be induced at early developmental stages by treatment with thyroid hormones. Having observed these contrasting programs of synapse development in two muscles of Xenopus laevis, it will be of interest to relate them to possible differences in the development of muscle function.

376.10 **Development of Command System Function During Transformation in the Sea Lamprey.** L. Margolin*, D. Kaufman* and J. Ayers. Marine Science Center, Northeastern University, East Point, Nahant, MA 01908

Although much is known about the fictive patterns elicited by electrical stimulation of brain command centers in lampreys, the behavioral significance of these motor patterns has received less attention. We have developed a preparation which allows quantitative analysis of movement parameters in response to command tract stimulation. We find, that although the command systems can evoke undulatory behaviors within the range of normal swimming, the descending control of the motor output in larvae differs quantitatively from that observed in recently transformed adults. Specimens were prepared for brain stimulation by a ventral longitudinal slit through the gill chamber which allowed the specimen to be pinned out by its gill arches. The head and brain case was then dissected away to expose the brain for focal extracellular stimulation (Brain Res 279: 238-240). Biphasic stimuli (ammocoetes, 5-50 hz, 7-46 μa; transformers, 5-20 hz, 20-35 μa) were delivered through double barreled glass microelectrodes inserted into the bilateral command tracts in the rhombencephalon.

In both larvae and transformed adults, the stimulation of this tract could evoke front to rear undulations within the range which characterizes normal swimming movements (Science 221: 1312-1314). In adults, high currents could sometimes evoke behavior similar in timing to other undulatory behaviors, but curvature was always much lower. In larvae, the motor output was typically (>60%) bilaterally symmetrical in curvature; whereas, in transformed adults, the motor output was typically (>90%) assymmetrical in curvature. In ammocoetes, increases in the stimulus current caused the period, intersegmental delay and intersegmental phase lag to decrease with no effect on curvature. Variation in the stimulus frequency was for the most part without effect. In contrast, in adult specimens, increases in stimulus current caused increases in period and delay with little effect on intersegmental phase lag and curvature. Increases in frequency caused decreases in period, intersegmental delay and curvature. We conclude that transformation induces developmental alterations in the efferent effects of this descending command system.

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376.11 LONG-TERM ADAPTATION OF CRAYFISH NEUROMUSCULAR SYNAPSES DOES NOT REQUIRE AN INCREASE IN THE ACTIVITY OF THE ALTERED SYNAPSES. G.A. Lnenicka and H.L. Atwood, Dept. of Physiology, University of Toronto, Toronto, Ontario, Canada M5S 1A8

We have previously reported that direct in vivo stimulation of a relatively inactive crayfish phasic motoneuron (the fast excitor to the claw closer muscle) results in greater fatigue-resistance of its neuromuscular synapses as well as a reduction in the initial transmitter release (Lnenicka & Atwood, 1985, J. Neurosci. 5:459-467). This effect, called long-term adaptation (LTA), requires 2h of stimulation per day for at least 3 days, lasts for at least 1 week, and cannot be produced in decentralized motor terminals (Lnenicka & Atwood, 1985, J. Neurobiol. 16 (2): 97-110). The ultrastructure of the motor terminals become more similar to tonic axon terminals as a result of chronic stimulation. We report here that increased activity of the altered synapses is not required for establishment of LTA.

To determine whether an increase in synaptic input to the central processes of the fast excitor is sufficient to produce LTA of peripheral synapses, tactile receptors on the biting surface of the claw which reflexively drive the fast and slow excitors were electrically stimulated in vivo. Although the slow excitor can be reflexively driven for hours in vivo, the fast excitor fails to spike after only a few trials, due to the high threshold for impulse production in this neuron (Wiens, 1976, J. Comp. Physiol. 112:213-233). The tactile receptors in one of the paired claws were electrically stimulated at 12 Hz in .75sec trains delivered every 2sec, 2h/day, for 1 week. Since the fast and slow excitors receive parallel sensory input (Wiens, ibid.), the effectiveness of the sensory stimulation was monitored by chronically recording the reflexively evoked myogram of the slow excitor. One day after the final stimulation period, fast excitor EPSP amplitudes were measured in experimental and contralateral control claws. Initial fast axon EPSP amplitudes in the experimental claw were significantly smaller and showed significantly less depression during 5 Hz stimulation, compared to the contralateral control claw - an effect similar to that produced by direct stimulation of the fast axon. These results suggest that depolarization of motoneuron's central processes can affect the properties of the neuromuscular synapses in the absence of motor action potentials. However, since the activity of the slow axon was increased during sensory stimulation, we presently cannot rule out the possibility that the increase in muscle fiber activity plays a role in initiating these synaptic changes in the fast excitor. The fact that the muscle fibers in which fast excitor EPSPs were measured are very weakly innervated by the slow excitor argues against this possibility. (Supported by NSERC and MRC of Canada).

376.12 CHANGES IN THE ORGANIZATION AND DENSITY OF SYNAPTIC CONTACTS IN MOTOR CORTEX DURING PRE- AND POSTNATAL LIFE OF RHESUS MONKEYS. N. Zecevic and P. Rakic. Section of Neuroanatomy, Yale University. School of Medicine, New Haven, CT 06510

We have studied the development of synapses in the motor cortex (Brodmann's area 4) of 16 rhesus monkeys at various embryonic (E) and postnatal ages. Two to four vertical EM probes consisting of 100-250 overlapping micrographs (14,000X) that spanned the thickness of the cortex were prepared for each animal. Synaptic density was calculated per unit volume of neuropil (excluding neuronal and glia cell bodies, blood vessels and extracellular space), and the synapses were categorized according to their morphology (symmetrical or asymmetrical) and location (on spines or on shafts). The length of synaptic contacts and the ratio of different types of synapses were recorded on each probe using an image analyzer. From the total of 115,000 synapses recorded, a sample of 11,000 were classified and analyzed statistically.

The first synapses to appear in the prospective motor cortex are observed at about E55 in the marginal zone (prospective layer I) and in the subplate zone situated beneath the developing cortical plate. Around midgestation synapses appear within the cortical plate itself. By E89 synaptic density is about 5/100um$^3$ and increases 8-fold during the last two months of gestation to reach 40/100um$^3$ at the time of birth. This period of rapid synaptogenesis continues until the 4th postnatal month when a peak density of 56/100um$^3$ is attained. Thereafter synaptic density declines gradually to a level of about 33/100um$^3$ in mature animals (3-5 years), and by the end of the second decade of life it diminishes even further to a level of about 20/100um$^3$. The decline in synaptic density is caused to a large extent by the loss of synaptic terminals rather than by the dilution of a constant synapse population due to growth in neuropil volume. Futhermore, the drop in absolute synaptic density can be attributed mainly to the elimination of synapses on dendritic spines: the population of synapses on dendritic shafts remains relatively constant. The predominant loss of axo-spinous synapses is reflected in a change in the ratio between asymmetrical and symmetrical synapses. This ratio is 4:1 at E112, 7:1 at birth, 24:1 during the 4th postnatal month, and again 7:1 in the oldest animals. While the mean length of synaptic junctions does not change significantly during development, the number of complex synapses - with multiple junctions - shows an overall increase from about 1% at E112 to about 10% during the second decade. Thus, the development of the complex synaptic architecture of the primate motor cortex involves overproduction, elimination, and rearrangement of synaptic junctions. These changes occur nearly simultaneously with similar events in visual, somatosensory, limbic, and association cortices (Bourgeois and Rakic, '83,'85; Rakic et al., '85).

376.13 REORGANIZATION OF THE SOMATIC REPRESENTATION IN MOTOR CORTEX FOLLOWING FORELIMB AMPUTATION IN NEWBORN RATS J.P. Donoghue, B.Z. Berger*, S. Ryan*, and J. Sanes. Center for Neural Science, Brown University, Providence, RI and Lab. Neurophysiology, NIMH, Bethesda MD.

Previous studies have found that damage to sensory receptors modifies the topographic organization of somatic representations in cerebral neocortex. However, the degree to which sensory reorganization is accompanied by corresponding modification of the cortical motor output is unknown. Accordingly, we examined the somatic representation in rat primary motor cortex (MI) subsequent to forelimb (FL) amputation. The FL was amputated at the shoulder joint on postnatal day 1. After a 1-6 month survival period, the organization of MI was mapped during ketamine anesthesia (100mg/Kg, i.p.), using intracortical microstimulation techniques (30msec trains of 200usec long pulses, 333Hz). Microelectrode penetrations were spaced from 100-500 um apart throughout the presumptive MI FL and adjacent areas. Current intensities up to 100uA were delivered 1.8 mm below the pial surface. Control maps were obtained from normal adult rats, normal littermates, or from MI ipsilateral to the amputation.

The size and location of the MI FL representation in controls generally agreed with previous studies in rat. Stimulation at less than 30 uA over a rostrocaudally elongated strip (mean area=4.7±1.2 mm$^2$; N=3) of frontal cortex elicited distal FL movements, most commonly, wrist extension and/or elbow flexion. Proximal (shoulder) movements were only observed by increasing levels of stimulation at distal FL sites in the caudal 2/3 of MI. In all 6 amputated rats, stimulation in the region of the presumptive FL representation produced discrete movements of muscles about the shoulder stump or scapula. Thresholds for proximal movements in amputated rats were less than 30uA (mean=19±6uA) at 37% of these sites, compared with 9% less than 30uA in controls. This proximal region overlapped the caudal region of the normal FL representation, but occupied a territory smaller than the usual FL area (mean area 2.7±1.1mm$^2$, N=5). In 5 of the 6 amputated rats, vibrissae movements were evoked by stimulation in rostral parts of MI that are normally occupied by the FL representation. Vibrissae movements were never observed in this same area in control animals. From these data we conclude that forelimb amputation in the newborn induces reorganization of MI so that part of MI normally influencing distal forelimb musculature is "redirected" to control novel muscle groups. Ongoing experiments will identify the extent of cortical and subcortical reorganization in these animals.

377.1 EFFECT OF GABAERGIC DRUGS ON THE YAWNING BEHAVIOUR OF THE RAT. E. Doger *, R. Urbá-Holmgren * and B. Holmgren * ( SPON: J.A. Roig ) Departamento de Ciencias Fisiológicas, Instituto de - Ciencias. Universidad Autónoma de Puebla, Pue., México.

It has been shown that cholinergic and dopaminergic mechanisms are involved in the genesis and regulation of yawning behaviour in mammals. An increment of the central cholinergic activity induces yawning behaviour, and an increment of the dopaminergic activity decreases its frequency. (Holmgren B., Urbá-Holmgren R., Acta Neurobiol. Exp., 40: 633-642, 1980). On the other hand, regulatory mechanisms between gabaergic and dopaminergic systems have already been described in some structures of the basal ganglia, (Mc Geer, et al., Canadian J. of Neurol.Sciences 11:89-99,1984).

Experiments were performed with the purpose of providing a first approach to the possible participation of gabaergic mechanisms in the regulation of this behaviour. The following drugs were used: gamma-acetilenic-gaba, progabide, bicuculline, methiodide; all drugs were injected i.p.

The behavioral observations were carried out in Sprague-Dawley male rats, 2-3 months old (n=10-12 per group) from a selectively bred subline to get spontaneus, highly yawning-rats. The results were analyzed with the U-Mann Whitney Test.

The dose-response curve of gamma-acetilenic-gaba shows an increase of yawning at 7 mg/kg ($p < 0.05$), other doses being ineffective. With progabide an increase in yawning behaviour was also obtained at 25 mg/kg ($p < 0.04$). Quite different results were obtained with bicuculline, which produced an inhibition of the yawning behaviour 3mg/kg ( $p < 0.007$), other doses being ineffective.

These results show that moderate increases of the gabaergic activity obtained by gamma-acetilenic-gaba and progabide produce an increment of yawning, while a gaba antagonist reduces yawning frequency. These data are consistent with the hypothesis that gaba inhibits the activity of dopaminergic neurons. Further studies are needed in order to obtain more information about the role of gaba, and the type of receptors involved in the modulation of yawning behaviour .

377.2 **EFFECT OF 1,4-BENZODIAZEPINES AND OTHER ANXIOLYTICS ON HARMALINE-INDUCED TREMOR.** W. J. Millard, Barbara R. Keith*, and Kristen C. Hughes*. Laboratory of Psychobiology, Mount Holyoke College, South Hadley, Massachusetts 01075-1462.

Harmaline is a ß-carboline that has established hallucinogenic and tremorgenic properties (Fuentes, J.A., & Longo, M.V.G., Neuropharmacol, 10:15, 1971.). Several ß-carbolines have been found to antagonize the spectrum of pharmacological effects of the 1,4-benzodiazepines, e.g., diazepam and chlordiazepoxide (Braestrup, C,, & Neilsen, M., Beta-carbolines and Tetrahydroisoquinolines, 227, 1982).

The objective of the present experiments was the characterization of the dose-response relationship for tremor induced by the acute administration of harmaline. The mechanism of action of harmaline was further studied in a second procedure in which the antagonism of harmaline-induced tremor by a chlordiazepoxide, Na pentobarbital, and Ro 15-1788, a 1,4-benzodiazepine antagonist.

The tremor induced by the acute administration of harmaline (1.0-100.0 mg $kg^{-1}$) was dose-dependent in C57BL/6J mice. The results of a second procedure indicated harmaline-induced tremor was antagonized by a 1,4-benzodiazepine, chlordiazepoxide (10.0-40.0 mg $kg^{-1}$).

The nature of the antagonism was specific as indicated by the absence of antagonism by chlordiazepoxide on tremor induced by oxotremorine (0.25 mg $kg^{-1}$), a compound having a cholinergic site of action.

377.3 **CENTRAL NICOTINIC BLOCKADE OF DIAZEPAM'S ANTICONFLICT EFFECTS IN THE RAT.** Gregory F. Heath* & Joseph H. Porter(SPON:M.L. Fine). Dept. of Psychology, VA Commonwealth Univ., Richmond, VA 23284.

Diazepam produces an increase in punished responding in conflict procedures in rats and other species that is predictive of its antianxiety activity. Heath and Porter (Eastern Psychological Assoc., 1985) reported that nicotine blocks the anticonflict effects of diazepam in rats tested in a modified Geller-Seifter conflict procedure(Psychopharmacologia, 1:482,1960). However, the muscarinic agonist, arecoline, did not block diazepam's anticonflict effects. These data suggest that this cholinergic antagonism of diazepam's anticonflict effects involves nicotinic, but not muscarinic, receptors. The present study examined the role of central versus peripheral nicotinic receptors in this blockade of diazepam's anticonflict effects.

Adult male rats (N=17) were maintained at 80% body weight and tested daily on a modified Geller-Seifter conflict procedure with a multiple fixed interval 1-min (food reward only) fixed ratio 1 (food plus scrambled foot shock) schedule. All rats were tested with the following drug conditions both alone and in combination: 0 and 4 mg/kg diazepam ; 0 and 1.4 mg/kg (-)-nicotine; 0 and 1 mg/kg mecamylamine (central nicotinic antagonist); and 0 and 1 mg/kg hexamethonium (peripheral nicotinic antagonist). Data on test days were converted to percent of the preceding control (non-drug) day. Separate Dunnett's tests were performed on conflict data (i.e., punished responding during FR components) for all drug conditions with 0 mg/kg diazepam and for all drug conditions with 4 mg/kg diazepam.

The increase in punished responding produced by diazepam (mean = 2034.6%) was significantly suppressed by nicotine (mean = 1181.9%). This effect of nicotine on diazepam was blocked by mecamylamine (mean = 2272.5%), but not by hexamethonium (mean = 635.8%). Mecamylamine in combination with diazepam resulted in a significantly greater increase in punished responding than diazepam alone (3399.5% vs. 2034.6%). Hexamethonium in combination with diazepam was not significantly different from diazepam alone (1663.3% vs. 2034.6%). In the absence of diazepam, mecamylamine produced a significant increase in punished responding as compared to vehicle (365.1% vs. 224.2%).

These data support our previous findings that nicotine blocks the anticonflict effects of diazepam. Further, it was demonstrated that this nicotinic blockade of diazepam's anticonflict effects appears to be mediated by central nicotinic receptors and that peripheral nicotinic receptors do not appear to be involved in this effect. One intriguing result was the synergistic effect of mecamylamine with diazepam, and the significant increase in punished responding produced by mecamylamine alone. These data suggest that mecamylamine may possess anxiolytic activity.

377.4 DOSE-RELATED EFFECTS OF DIAZEPAM ON NOISE-INDUCED POTENTIATION OF THE ACOUSTIC STARTLE REFLEX: DISCLOSURE OF SEVERAL UNDERLYING MECHANISMS. A. T. Sullivan* and C. K. Kellogg. Dept. of Psychology, University of Rochester, Rochester, NY 14627.

The noise potentiated acoustic startle reflex (ASR) has been utilized to elucidate the effects of prenatal and adult exposure to diazepam (DZ). The ASR was induced by a 120 dB tone burst against a background noise (centered about 1 kHz) of 30, 50, 70, or 90 dB. In the prenatal study, pregnant dams (Long Evans strain) were administered DZ at doses of 1.0, 2.5, or 10 mg/kg per day during gestational days 13-20. Previous work indicated that prenatally exposed rats had a reduced ASR and background noise did not facilitate the ASR (Sci., 207:205, 1980). In the present prenatal study, exposed offspring were tested in adulthood (70-90 days), and all animals (exposed and uninjected controls) demonstrated similar amounts of potentiation of the ASR. However, significant differences in the baseline ASR were apparent. Rats exposed to DZ at 2.5 mg/kg were hyperresponsive to the startle stimulus, rats exposed to DZ at 10.0 mg/kg were hyporesponsive, and rats exposed to DZ at 1.0 mg/kg showed no differences as compared to uninjected controls. Furthermore, combined exposure to DZ (2.5 mg/kg) and the benzodiazepine antagonist RO 15-1788 (10 mg/kg) prenatally did not prevent the hypersensitivity to the ASR observed in rats exposed to 2.5 mg/kg DZ alone. These observations suggested that DZ given prenatally may intereact with multiple receptor subtypes.

Administration of DZ to normal adults in cumulative doses of .25, .75, and 2.25 mg/kg induced a dose-related decrease in noise potentiation of the ASR with no effect on basal ASR. If RO 15-1788 was given concurrently with DZ, the inhibition by DZ was enhanced. Further study demonstrated that a single dose of DZ (2.5 mg/kg) induced a 73% inhibition of noise potentiated ASR. This effect was reversed by a subsequent injection of RO 15-1788 but not by vehicle. However, a single dose of DZ at .25 mg/kg induced only a 16% reduction in potentiation which was enhanaced to 73% and 72% when animals were given either RO 15-1788 or vehicle. Basal ASR increased in the last test. The low dose of DZ may engage mechanisms which are not sensitive to the central antagonist. Initial studies have shown that neither the triazolopyridizine CL 218-872 nor methyl-betacarboline-3-carboxylate alter potentiation or basal ASR; hence, analysis of noise potentiated ASR may elucidate mechanisms of action of DZ and related compounds not disclosed by other paradigms. Supported in part by grant MH 31850.

377.5 ALCOHOL AND AGGRESSIVE BEHAVIOR IN RATS: INTERACTION WITH BENZODIAZEPINES. K.A. Miczek. Dept. of Psychology, Tufts Univ., Medford, MA 02155.

The discovery of benzodiazepine receptors and the development of drugs that selectively and specifically interact with these receptors provides new avenues to investigate the mechanisms by which alcohol alters behavior. (1) The behavioral profiles of alcohol and diazepam are similar; particularly intriguing are the dose-dependent bidirectional effects of both drugs on aggressive behavior. (2) Moreover, alcohol and diazepam appear to add to each other's aggression-enhancing and -suppressing effects, when given concurrently. (3) Whether or not specific benzodiazepine receptor blockade may alter alcohol's effects on aggression remains unresolved.

In several series of experiments pharmacological manipulations of benzodiazepine receptors were performed in order to study their role in alcohol effects on aggressive behavior in rats. The resident males of 12 independent rat colonies were confronted twice weekly with an intruder male. The resident's attack, threat, pursuit behavior, aggressive postures and grooming as well as nonaggressive rearing, walking and grooming were encoded into computer files while viewing the videorecords of the resident-intruder encounters. In addition, the intruder's defensive, submissive and escape reactions were measured.

The frequency of attack bites and the display duration for aggressive posture was significantly increased by either a low diazepam (0.3, 1.0 mg/kg, i.p.) or a low alcohol dose (0.3 g/kg, p.o.). In the presence of a fixed low alcohol dose, either 0.1 or 0.3 g/kg, the dose-effect curve for diazepam on the resident's aggressive behavior was shifted to the left, indicating additive effects on both the aggression-enhancing and -decreasing effects of the two compounds. It will be important to determine how concurrent administration of the specific benzodiazepine receptor blocker Ro15-1788 modulates the aggression-enhancing and -suppressing effects as well as the sedative effects. At present, it remains unclear at what level alcohol and diazepam in their respective mechanisms of action interact in order to produce not only similar, but also additive effects on aggressive behavior.

377.6 PUNISHMENT ATTENUATING EFFECTS OF PENTOBARBITAL ARE NOT RATE-DEPENDENT. S.I. Dworkin, T. Miyauchi* and C. Bimle*. Psychiatry Research Unit, Departments of Psychiatry and Pharmacology, Louisiana State University Medical Center, Shreveport, LA 71130.

Drugs used clinically for their anxiolitic or sedative properties typically increase punished responding. These drugs, however, also increase low rates of responding that are not suppressed by punishment. Therefore, comparable rates of punished and unpunished responding are necessary to investigate potential specific punishment-attenuating effects of these agents. This study investigated the effects of pentabarbital on such a baseline.

Five pairs of littermate rats were trained on either a random-ratio 50 or a yoked random-interval food presentation schedule. The random-ratio subject received a food pellet after completing a randomly select number of responses (average 50). A reinforcer was presented to the yoked random-interval subject for each response that immediately followed a reinforcer delivery to the subject on the ratio schedule.

This procedure resulted in high rates of responding maintained by the subject on the ratio contingency and low rates by the subject on the interval schedule. However, the reinforcer density was the same for both subjects. A random ratio schedule of electric footshock presentation was then added to the random-ratio food presentation schedule. The intensity of the shock was adjusted to suppress responding to rates comparable to those maintained by the yoked-interval contingency. This additional contingent resulted in similar rates of punished and unpunished responding and nearly equivalent reinforcement density. Moderate doses of pentobarbital (10 mg/kg) increased the low rates of punished responding but did not increase similar rates of unpunished responding. Larger doses (17 mg/kg) decreased responding maintained by both contingencies. These data demonstrated that pentobarbital selectively increased punished responding.

377.7 DOES DIAZEPAM CAUSE SEIZURES IN INFANT RATS? J.W. Smythe,* C.L. Ryan* and B.A. Pappas* (SPON: T. Tombaugh). Unit for Behav. Med. & Pharmacol., Carleton Univ., Ottawa, Ont., K1S 5B6.

This laboratory has previously shown (Pappas & Walsh, Pharmacol. Biochem. & Behav., 1983) that benzodiazepines (BZs) produce a behavioural syndrome in infant rats characterized by leg paddling and wall progression. Barr and Lithgow (Nature, 1983) also demonstrated this syndrome, which they suggest may indicate "behavioural convulsions." If BZs have intrinsic convulsogenic activity in rat pups, they would not block the effects of a known chemical convulsant. The following study investigated this hypothesis by determining if diazepam (DZP) blocks pentylenetetrazol- (PTZ) induced seizures in rat pups.

Four groups of seven-day-old Wistar rat pups were used to examine the putative convulsogenic activity of DZP. The first group was injected subcutaneously with 0.5 mg/kg DZP while a second group received 65 mg/kg PTZ. A third group was administered a pretreatment of 0.5 mg/kg DZP, followed 30 minutes later by an injection of PTZ. A fourth group received only saline, and served as the controls. The groups were assessed for the overt behavioural manifestation of paddling and wall progression, head tremor, and body tremor.

DZP alone elevated paddling and wall progression scores, but did not alter head and body temor scores. PTZ alone did not affect paddling and wall progression, but significantly elevated head and body tremor scores. The combined DZP + PTZ treatment produced the same elevated paddling and wall progression as did DZP alone. Head tremor scores were significantly reduced, however, while body tremor scores were reduced, but not significantly.

These results indicate that DZP reduces the effect of PTZ and thus seems to be an anticonvulsant in young pups. If BZ-induced paddling and wall progression are behavioural manifestations of brain seizures, then at the very least these seizures are markedly different than those produced by PTZ. Alternatively, these behaviours do not reflect brain seizures. Confirmation of one or the other of these alternatives requires analysis of EEG activity after BZ administration. Preliminary investigations indicate that DZP may flatten the EEG and cause interspersed spike activity in rat pups. In contrast, PTZ causes marked and virtually continuous spiking. Further EEG data will be presented at the meeting. (Supported by NSERC)

377.8 EFFECTS OF PSYCHOACTIVE DRUGS ON A DRL TASK IN CATS. M. Curtis*, P. Saxton, J. Siegel. (Spon: A. M. Granda). Institute for Neuroscience, University of Delaware, Newark, DE, 19716.

In the first phase of a series of experiments designed to study central nervous system inhibitory mechanisms, the effects of psychoactive drugs on a differential reinforcement of low rate of response (DRL) operant task was determined in cats. Six cats were trained to perform on a DRL food-motivated bar-press schedule which involves an active behavioral inhibitory process. DRL cats served as their own drug injection controls. Drug effects were also contrasted with two cats trained on a fixed interval (FI) operant schedule, which uses the same motor patterns but does not involve an active inhibitory mechanism.

Using a blind procedure, fasted animals were dosed once a week with one of three drugs. Imipramine at 6.0 - 9.5 mg/kg was given i.p. 45 min before testing, chlordiazepoxide at 4.0 mg/kg i.p. 15 min before testing, and haloperidol at 0.2 mg/kg i.m. 2 hrs before testing. Minimal CNS side effects noted such as slight ataxia were recorded up to 3 hrs after dosing. Performance indices (errors, reinforcements, inter-response times, and perseveration) were tabulated for each 5 min period in a 30 min test session by a computer. Errors on a DRL schedule were made when the animal failed to withold a bar press for a specified period of time (e.g. 15 sec). Reinforcements (liquid mash reward) were received when the animal bar-pressed at the correct time.

All three drugs impaired performance using the DRL paradigm. Imipramine, a tricyclic antidepressant, increased DRL errors without significantly altering number of reinforcements but decreased FI extra presses. Haloperidol, a butyrophenone neuroleptic, increased DRL errors, decreased FI extra presses, and caused pausing in bar-pressing activity which resulted in decreased reinforcements. Chlordiazepoxide, a benzodiazepine anxiolytic, dramatically increased both DRL errors and FI extra presses. Disinhibition that resulted from chlordiazepoxide treatment could not be attributed to a response rate-dependent effect since both DRL and FI response rates were accelerated. The differential effects of these drugs provide clues about the interaction of neurotransmitter systems involved in inhibition on a DRL task, and serve as the basis for direct brain infusions of more selectively acting agents.

377.9 DISCRIMINATIVE STIMULUS PROPERTIES OF CENTRALLY APPLIED MIDAZOLAM: GENERALIZATION FROM PERIPHERAL TRAINING CONDITIONS. R.R. Griffiths*, M.J. Kuhar and N.E. Goeders (SPON: S. Lukas). Departments of Neuroscience and Behavioral Biology, Johns Hopkins University School of Medicine and Laboratory of Neuroscience, NIDA Addiction Research Center, Baltimore, MD 21205.

The discriminability of a number of benzodiazepines (BZD) has been demonstrated in studies in which rats were trained to discriminate the peripheral administration of a BZD from vehicle. The autoradiographic visualization of BZD receptors has demonstrated an uneven distribution of these receptors in the rat brain, with high densities localized in the cortex, amygdala, hypothalamus and cerebellum (Young and Kuhar, J. Pharm. Exp. Ther., 212, 337, 1980). This investigation was designed to determine whether the central application of a BZD into discrete brain regions would occasion drug-appropriate responding in rats trained to discriminate peripherally administered BZD.

Male Long Evans hooded rats were trained to discriminate the triazolobenzodiazepine, midazolam (1.0 mg/kg, i.p.) in a two-lever drug discrimination procedure (Ator and Griffiths, J. Pharm. Exp. Ther., 226, 776, 1983). Food delivery depended on 10 consecutive responses on one lever in sessions preceded by an injection of the training dose of midazolam and on 10 consecutive responses on the other lever after no drug. All rats reliably completed 96-100% of total session responses on the appropriate lever with no response runs of the length required for reinforcement on the incorrect lever prior to the first pellet delivery. Following the completion of dose-response curves with i.p. drug delivery, the rats were stereotaxically implanted with unilateral guide cannulae into either the medial prefrontal cortex or posterior division of the basolateral amygdaloid nucleus and were allowed two weeks to recover. The surgical procedures did not affect response rates or i.p. midazolam discrimination. Intracranial microinjections of low doses of midazolam (0.1 to 10 nmol) did not occasion drug-lever responding in animals with cannulae implanted into either brain area. However, rats responded on the drug lever (>96%) when microinjected with higher concentrations (50 to 200 nmol) into the medial prefrontal cortex. Microinjections of similar doses of midazolam into the amygdala did not occasion consistent drug-lever responding. Injections of higher doses (>400 nmol) into either brain locus resulted in pronounced ataxia and little or no responding on either lever.

The results of this investigation suggest that neuronal systems associated with the discriminative stimulus properties of peripherally administered midazolam can be activated directly through the discrete application of the drug into the medial prefrontal cortex. (Supported by USPHS Grants DA 01147, MH 09111, DA 00266, MH 00053 and the McKnight Foundation).

377.10 DIAZEPAM DISRUPTS SEXUAL, AGGRESSIVE AND GROOMING BEHAVIORS IN THE RAT. N. Shanks* and T.B. Wishart, Department of Psychology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, S7N 0W0.

The behavioral effects of diazepam on normal rats in a naturalistic environment were studied during acute and chronic administration of the drug and during withdrawal. An ethological rating technique (modified from Silverman, A.P., Brit. J. Pharm., 24:579, 1965) consisting of 29 distinct behavioral components was used to analyze the behavior of male rats treated chronically (28 days) with diazepam (2 mg/kg) when housed continuously with a female conspecific, or when combined with a novel female.

When housed continuously with a female rat, diazepam treated males were observed to sleep more, explore less, and engage in fewer types of behaviors generally during the first few days of treatment. As treatment progressed, tolerance developed to these sedative effects of diazepam. Treated animals initiated fewer sexual behaviors, but were both more aggressive and more submissive than controls. Grooming behaviors were virtually absent in the drugged animals throughout the 28 day treatment phase.

When paired with a novel, sexually receptive female, males treated with diazepam were again found to be both more aggressive and submissive. They also displayed qualitatively different sexual behaviors in that the copulatory sequence occurred less frequently, lasted longer, and was incomplete on many occasions. Once again, diazepam completely blocked the occurrence of grooming.

Most of the behavioral differences between controls and experimental animals had disappeared by the end of the two-week withdrawal period.

The results indicate that diazepam has effects on a wide spectrum of behaviors in the normal animal; this has implications for the possible side effects of this drug in man.

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377.11 ELECTROACUPUNCTURE INDUCED ANALGESIA AND GAMA - AMINO-BUTYRIC ACID (GABA) IN MAMMALIAN CENTRAL NERVOUS SYSTEM. Sumita Chakraborty¶ A. Ganguly*and M.K.Poddar. Department of Biochemistry, University of Calcutta, 35 B. C. Road, Calcutta-700 019, INDIA.

Acupuncture, the ancient Chinese medical treatment is being widely used for curing various diseases. It is also known that acupuncture or electroacupuncture developes analgesia. In the present investigation, the effect of electroacupuncture(EA) induced analgesia on GABA system of rat central nervous system(CNS) have been studied. Adult male albino rats(120 - 140g) exposed to EA(Frequency, 10 Hz/sec; Intensity, 1 volt) were sacrificed by cervical dislocation at different time periods (2, 10 and 15 min.) during EA induced analgesia(onset, optimum and duration respectively) and at its post periods(10 and 30 min.). GABA level was increased during post acupuncture analgesia in thalamic(63.5%-101.3%) and hypothalamic(100.8%-77.9%) region of the brain. No significant change in GABA level was observed in pons-medulla, cerebral cortex and spinal cord during and after EA. Glutamic acid decarboxylase(GAD) activity was increased at optimum period of EA(10 min.)induced analgesia in only thalamus(29.3%) and at post EA induced analgesia both in thalamus(10-41.3%) and hypothalamus(17.5%-65.0%) No significant change was observed in other regions of CNS during or after EA. GABA-transaminase(GABA-T) activity was increased at the time of onset(116.3%), optimum (81.6%) and at 30 min after EA induced analgesia(263.2%) in pons-medulla. In thalamus GABA-T activity was increased at the optimum(78.3%) and at 30 min after EA induced analgesia(23.5%). No appreciable change in GABA-T activity was found in other regions of the CNS(hypothalamus, cerebral cortex and spinal cord) during and after EA. These results suggest that the GABAergic system in pons-medulla and thalamic regions of the brain is involved during EA induced analgesia; whereas in post period of the EA induced analgesia the GABAergic system of the thalamic and hypothalamic regions are affected. (Supported by Indian Council of Medical Research, New Delhi, India).

377.12 COMPARISON OF DRUG VS. DRUG DISCRIMINATION PARADIGMS USING CHLORDIAZEPOXIDE VS. CAFFEINE AND CHLORDIAZEPOXIDE VS. PENTYLENETETRAZOL. F.A. Holloway, R.C. Michaelis, A.M. Holohean*, and G.A. Hunter*. Dept. of Psychiatry and Behavioral Sciences, Univ. of Oklahoma Health Sciences Center., Oklahoma City, OK 73190.

Two drug vs. drug discriminations were trained in two separate groups of rats, pitting chlordiazepoxide (CDP) versus caffeine (CAF) or pentylenetetrazol (PTZ). Subjects were trained under a VR 10 schedule of reinforcement. Tests consisted of two minute sessions under extinction conditions, during which the total number of responses and the percent of responses emitted on each lever were monitored. Acquisition of both discriminations proceeded more quickly than acquisition of the respective drug vs. saline discriminations. In addition, compared to drug vs. saline discriminations, drug vs. drug training produced a greater percentage of responses on the drug-appropriate lever when subjects were tested after low doses of the training drugs.

Testing subjects in the undrugged (saline) state revealed interesting differences between the two paradigms. After several days of no drug exposure, saline produced approximately equivalent responding on both levers in both groups. However, response rates were drastically reduced in the CDP/CAF discriminators only. In CDP/PTZ discriminators, saline tests at various times after large doses of CDP produced a predominance of CDP lever responding for approximately 12 hours, followed by a predominance of PTZ lever responding. The period of predominantly PTZ lever responding could also be precipitated at shorter postinjection intervals by injection of RO 15-1788. A similar temporal pattern of change in response distribution was not seen in the CAF/CDP discriminators.

These findings suggest that the CDP/CAF discrimination represents two orthogonal stimuli, and that testing under saline presents a third orthogonal stimulus which is not perceived as being similar to either of the training stimuli. In contrast, the discriminative stimuli generated by CDP and PTZ appear to lie at opposite ends of an affective continuum, with the undrugged (saline) state capable of illustrating the dynamic biphasic changes in subjective stimulus state which follow injection of large doses of these training drugs.

377.13 CHLORDIAZEPOXIDE VS. CAFFEINE DRUG DISCRIMINATION IN THE RAT. A.M. Holohean*, F.A. Holloway, R.C. Michaelis, and G.A. Hunter*. Dept. of Psychiatry and Behavioral Sciences, Univ. of Oklahoma Health Science Center, Oklahoma City, OK 73190.

Drug. vs. drug (DD) discriminations have most frequently employed drugs whose stimulus properties have at least one aspect in common. Little parametric data is available concerning DD discrimination using contrasting drugs. In this study, a discrimination was trained between caffeine (CAF) and chlordiazepoxide (CDP), which produce different discriminative stimuli and interact antagonistically in several behavioral and biochemical tests. Rats were trained to discrminate 32.0 mg/kg CAF from 5.0 mg/kg CDP (i.p. 30 min prior to session) using a VR 10 schedule of food reinforcement. Tests consisted of two minute sessions under extinction conditions. The percent of responses made on each lever and the total number of responses made in the two minute session were monitored.

The CAF/CDP discrimination was acquired more quickly than the corresponding drug vs. saline discriminations. In addition, subjects showed higher percentages of drug-appropriate lever pressing at low doses of the training drugs than are seen in drug vs. saline paradigms. Subjects showed significant ( > 80%) generalization to the training cues after only 8.0 mg/kg CAF and 0.625 mg/kg CDP, respectively. Response rates were minimal after injection of saline or low doses of the training drugs.

Both accuracy and response rate varied in some tests depending upon whether the previous day's training session had involved CAF or CDP injection. In those subjects who maintained responding at low doses, greater percentages of CAF lever presses were seen after low doses of CAF when tests were run following a CDP injection day versus a CAF injection day. In addition, overall response rates during some CAF tests were higher following a CDP injection day versus a CAF injection day. When training drugs were administered together, a significant percentage of CDP lever responding ( > 77%) was seen after 32 mg/kg CAF plus doses as low as 1.25 mg/kg CDP.

The dramatic decrease in response rates during saline tests suggests that CAF and CDP produce orthogonal discriminative stimuli, and that the saline stimulus is a third orthogonal stimulus which does not generalize to either. In addition, results showed that training drug injections could influence performance on tests for a period of time which far exceeded the expected presence of the drug in the brain. These data suggest that contrasting DD discrimination produce quantitatively and qualitatively different generalization curves for the training drugs than that seen wth the corresponding drug-saline procedures.

377.14 RECOVERY OF FUNCTION AFTER BRAIN DAMAGE: SEVERE AND CHRONIC DISRUPTION BY DIAZEPAM. T. D. Hernandez*, T. Barth* and T. Schallert. Dept. of Psychology, Univ. of Texas at Austin, TX 78712.

Unilateral damage to anteromedial cortex (AMC) in rats causes a somatosensory asymmetry that is readily quantified using tactile extinction tests (originally designed to assess sensory function after damage to nigrostriatal dopaminergic neurons: Schallert et al., *Pharm. Biochem. Behav. 19*: 753, 1983; Schallert & Whishaw, *Behav. Neurosci. 98*: 518, 1984; Barth et. al., *Neurosci. Abstr. 10*: 1146, 1984). Recovery (a gradual reduction in the magnitude of sensory asymmetry) occurs consistently by about a week after surgery. The present study was conducted to determine whether daily postoperative exposure to diazepam interferes with this recovery. So that sedation would not be a factor, all behavioral tests were conducted *before* daily drug treatment. The dose used (5 mg/kg) was well below that necessary to affect sensorimotor behavior in control animals at the time of testing.

The results were that diazepam completely blocked recovery in all animals. Even following discontinuation of diazepam (after 22 days), the sensory asymmetry continued unabated. Up to 3 months after surgery (the duration of our observations), the magnitude of the asymmetry remained just as severe as it was on the first postoperative day. In contrast, undrugged (vehicle-treated) animals with AMC lesions recovered by 8 days. Apparently, the recovery process failed to occur in the drugged animals. Sham operated animals given diazepam or vehicle showed no postoperative asymmetry.

In a second experiment, rats with AMC lesions were given their first dose of diazepam at least three weeks *after* they had recovered from sensory asymmetry. A mild asymmetry was reinstated in a few of the animals, but only transiently (1-2 days) despite continuous drug treatment.

The data indicate that there may be a critical period after brain damage in which the recovery process is particularly vulnerable to diazepam. The effects of other agents on recovery are currently being evaluated, including drugs with anti-convulsant properties commonly used clinically to prevent seizures following brain damage.

377.15 INFLUENCE OF TESTING CONDITIONS ON THE ASSESSMENT OF DRUG EFFECTS IN A RAT OPEN FIELD TEST. F.O. Risinger*, W.M. Bourn, and R.L. Garrett*. Northeast Louisiana University, Division of Pharmacology and Toxicology, Monroe, LA 71209.

The characteristics of the testing environment have been shown to influence open field behavior. The purpose of this study was to examine the effects of different levels of illumination and sound on a multivariate assessment of drug effects in a rat open field test.

Adult female Sprague-Dawley rats were randomly assigned to 1 of 4 experimental conditions and 1 of 3 drug treatment groups (N = 20 per group). They were observed in an open field chamber (90 x 90cm) 20 minutes after dosing (i.p.). The open field observations lasted 15 minutes with ambulation, rearing, defecation, inner square penetration, and grooming recorded. The testing conditions consisted of either high (70fc) or low (.5fc) illumination with either high (100db tone) or low (5db background noise) sound. Drug treatment consisted of either chlordiazepoxide (3 mg/kg), pentylenetetrazol (20mg/kg), or saline. As a further characterization of locomotor effects, an additional experiment was conducted using activity cages. Large and small movements were measured under the same drug treatments as previously stated.

Multiple discriminant analysis of the open field data indicated that the greatest separation of drug treatment groups occurred in the low illumination conditions. For each of the low illumination conditions, two significant ($p < .01$) discriminant functions were derived. Within group classification was 83% for either low illumination condition. For both of the high illumination conditions, only one significant discriminant function was derived. Under the high illumination-high sound condition, 72% of the animals were correctly classified while under the high illumination-low sound condition, 68% of the animals were correctly classified. In the activity cages, both drug treatments produced a decrease ($p < .01$) in small movements while chlordiazepoxide produced a decrease ($p < .05$) in large movements.

These results indicate that testing conditions are a factor in the relative sensitivity of open field procedures when used to identify drug effects. Also, the locomotor effects of a drug are partially dependent on the experimental situation and instrument.

378.1 ATROPINE POTENTLY INHIBITS THE ONSET BUT NOT THE REEMERGENCE OF OVARIAN STEROID-INDUCED MATERNAL BEHAVIOR. C.A. Pedersen, J.D. Caldwell*, P.J. Brooks* and A.J. Prange, Jr. (SPON: R.L. Glasser) Department of Psychiatry, Biological Sciences Research Center and the Neurobiology Curriculum, University of North Carolina, Chapel Hill, NC 27514

The role of classical neurotransmitter systems in MB has been little studied. In this investigation we examined the effect of muscarinic and nicotinic antagonists on the onset and reemergence of MB.

Nulliparous Sprague Dawley rats were ovariectomized under ether anesthesia and treated with 17-β estradiol (E) and progesterone (P) for a two week period. Thirty minutes prior to introduction of three 1-5 day old rat pups some nullipara received ICV various doses of atropine sulfate (AS) or hexamethonium bromide (HB) in normal saline (NS) or NS vehicle alone. Nullipara receiving no ICV treatment before introduction of pups and primipara were allowed 4-5 days of mothering experience before being separated from pups for 24 hrs. Thirty minutes prior to reintroduction of three 1-5 day old pups primipara and nullipara received ICV various doses of AS or NS vehicle alone. Behavioral responses were recorded during each of the first six hrs and the twenty-fifth hr after introduction of test pups.

In naive nullipara, all doses of AS (0.75, 1.5, 3.0, 6.0, 12.5 and 25 µg) significantly lowered the incidence of full maternal behavior (FMB) in the first hr of pup contact compared to NS. AS doses of 6.0, 12.5 and 25 µg significantly lowered the cumulative incidence of FMB in each of the first 3-6 hrs of pup contact. In contrast, a dose of HB equimolar to 12.5 µg AS had no significant effect on the cumulative incidence of FMB in any hr of observation. In nullipara and primipara allowed 4-5 days of MB experience 12.5 µg of AS did not significantly lower cumulative incidences of FMB in any hr of observation while 25.0 µg of AS significantly lowered the incidence of FMB compared to NS in the first hr of pup contact alone. AS doses of 12.5 and 25.0 µg produced significantly lower cumulative incidences of FMB in E + P treated nullipara with no prior pup contact compared to nullipara and primipara with prior MB experience over the first three hrs (12.5 µg AS) or two hrs hrs (25.0 µg AS) of pup contact.

We conclude that muscarinic but not nicotinic cholinergic neurotransmission plays a critical role in the ovarian steroid-induced onset of FMB but not in the reemergence of established FMB.

378.2 COCAINE EXPOSURE INCREASES SUBSEQUENT BEHAVIORAL RESPONSE TO NICOTINE. R.L. Hakan* and C. Ksir. Dept. of Psychology, University of Wyoming, Laramie, WY 82071.

Rats given daily injections of nicotine develop an increased stimulant response to nicotine which is concomitant with an increase in the number of CNS nicotinic cholinergic receptor sites (Ksir, Hakan, Hall & Kellar, Neuropharmacology, 1985). Amphetamine, cocaine and morphine have each been reported to produce a similar type of behavioral sensitization. We now report that in three separate experiments, rats preexposed to cocaine for five days showed an increased behavioral activation to a test dose of nicotine given on day 6.

In one experiment, 12 rats were placed in the photocell test cages each day for a 1-hour adaptation period. Then six rats received saline and six received 5 mg/kg cocaine (i.p), after which activity was monitored for another hour. This was repeated for five days. On the sixth day all rats received a test dose of 0.2 mg/kg nicotine (s.c.). The rats that had been exposed to cocaine showed greater activity during the first ten min after nicotine than the rats previously given saline ($t$=4.08, df=10, $p<.01$).

Experiments in which rats were exposed to daily doses of morphine (5 mg/kg) or d-amphetamine (0.5 or 1.0 mg/kg) found no differences in nicotine effects on the test day. Thus, this effect is not common to all drugs that might produce behavioral sensitization.

In another experiment, rats were given preexposure injections of cocaine or saline in their home cages. Again there was significantly greater activity during the first ten minutes after nicotine in the group preexposed to cocaine ($t$=2.9, df=10, $p<.01$). In a third cocaine experiment, rats were given either saline or 10 mg/kg cocaine each day for five days in their home cages. Again the cocaine preexposure group showed more activity following nicotine ($t$=2.69, df=9, $p<.025$).

Another group of rats was given an initial test dose of 5 mg/kg cocaine in the test cages, after which they received five once-daily injections of 0.2 mg/kg nicotine followed by a second test dose of cocaine. The response to cocaine on the post-nicotine test was not significantly greater than the response on the pretest (in fact the trend was for a reduced effect). This indicates that the cocaine-nicotine interaction is a one-way preexposure effect.

The mechanism by which cocaine preexposure alters subsequent nicotine response is unclear. Preliminary binding results indicate that cocaine does not bind to nicotinic sites nor does cocaine preexposure increase nicotinic receptor numbers.

378.3 NICOTINE BEHAVIORAL EFFECTS IN A FISH, PIMEPHALES PROMELAS (fathead minnow). D.A. Scratchley*, R.L. Hakan* and C. Ksir (SPON: M. Renner). Dept. of Psychology, University of Wyoming, Laramie, WY 82071.

Nicotine has been shown to produce increased locomotor activity in rats, as measured in photocell activity cages (Ksir, Hakan, Hall & Kellar, Neuropharmacology, 1985). In the current experiments minnows were placed in 20 x 20 cm testing containers. The floor of each container was marked so that 4 equal quadrants were defined. Twelve of these containers were simultaneously monitored from above by a video camera. Each tank contained 1500 ml of dechlorinated tap water at room temperature. After a 10 min drug-free adaptation period, nicotine (1 mg/l), mecamylamine (5 mg/l) or hexamethonium (10 mg/l) was added to some of the containers. Behavior was then recorded for ten minutes on a videocassette. Subsequently, the activity of each fish was indidivually scored in terms of quadrant entries.

Fish exposed to no drug made an average of 28.8 quadrant entries (+/- 3.9) in the 10-minute test period. Fish exposed to nicotine made almost ten times as many quadrant entries (247.5, +/- 11.8). The fish given mecamylamine and nicotine showed no significant increase in activity relative to controls, whereas fish given hexamethonium plus nicotine showed a large increase in activity similar to that seen with nicotine alone. Mecamylamine and hexamethonium are both classical nicotinic antagonists, but hexamethonium does not readily cross the mammalian blood-brain barrier. The current results imply that the minnow's blood-brain barrier also limits uptake of hexamethonium, and that this dramatic behavioral effect of nicotine is mediated by drug action within the central nervous system.

378.4 CHOLINERGIC INVOLVEMENT IN THE BEHAVIORAL ACTIONS OF FORMETANATE, A FORMAMIDINE PESTICIDE. V.C. Moser and R.C. MacPhail*. Neurotoxicology Division, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711.

Formetanate (FMT) is a formamidine acaricide/insecticide which also has a carbamate moiety in its molecular structure. FMT-induced lethality is reportedly due to an inhibitory action on acetylcholinesterase (AChE, EC 3.1.1.7; see Knowles and Ahmad, Pest. Biochem. Physiol. 1:445-452, 1972). We set out to determine its monoamine oxidase (MAO; EC 1.4.3.4) inhibitory properties (a common feature of formamidines) as well as its AChE inhibitory actions and to examine in rats the neurochemical basis for the sublethal, behavioral effects of FMT.

In rat brain homogenates, FMT inhibited AChE at concentrations $\geq$0.03 µM, with a IC50 of 0.14 µM. FMT also inhibited MAO in vitro, and was selective for type A MAO. Inhibitory concentrations were $\geq$10 µM with an IC50 of 86.2 µM. Type B MAO was slightly inhibited only at higher concentrations (>1 mM). Thus, FMT was a more potent inhibitor of AChE in vitro, even though it did efficaciously inhibit MAO-A.

For in vivo measurements, rats were dosed i.p. either with FMT (1 mg/kg) and sacrificed 0.5-24 hr post-injection (N=5/time point), or with FMT (0.03-5.6 mg/kg; N=5/dose) and sacrificed 1 hr later. AChE activity was inhibited maximally at 0.5-1 hr post-injection and had returned to control levels at 3 or more hr post-injection. The ED50 for AChE inhibition was 0.9 mg/kg. MAO-A and MAO-B activities were not inhibited by FMT in vivo.

Horizontally- and vertically-directed motor activity were decreased in rats (N=12) receiving $\geq$1 mg/kg FMT 20 min before testing. In rats (N=7) trained using milk reinforcement to lever-press under a multiple fixed-interval 1-min fixed-interval 5-min schedule, FMT 0.56 mg/kg (5 min before the 55 min session) produced a pronounced suppression of response rates. Injections of scopolamine (0.1 mg/kg) and methyl-scopolamine (0.1 mg/kg) 15 min before the FMT dose blocked the response rate suppression, whereas neither mecamylamine (2 mg/kg) nor hexamethonium (2 mg/kg) did. These data indicate that the behavioral effects of FMT on schedule-controlled responding are primarily mediated by central and peripheral muscarinic receptors. The similarity in the dose ranges for AChE inhibition and for disruption of both motor activity and operant responding, along with the ability of muscarinic antagonists to block the effects of FMT on operant responding, suggest that the behavioral effects of FMT are closely associated with inhibition of AChE.

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378.5 EFFECTS OF INTRACRANIAL APPLICATION OF VARIOUS CHOLINERGIC DRUGS AND ESTROGEN ON LORDOTIC BEHAVIOR IN LONG EVANS RATS. L.S.Kaufman, D.W. Pfaff and B.S. McEwen. Labs of Neuroendocrinology and Neurobiology and Behavior, The Rockefeller University, New York 10021

Previous experiments have demonstrated that lordotic behavior in albino rats is mediated, in part, by cholinergic mechanisms (Clemens et al., Pharm. Biochem. Behav. 13:81, 1980; Kaufman et al., Neurosci. Abstr. 10, 1984). The goal of the present investigation was to study cholinergic mechanisms of lordotic behavior in the basomedial hypothalamus. Eighteen Long Evans female rats were ovariectomized and implanted under anaesthesia with bilateral cannulae that were stereotaxically placed in the basal hypothalamus; 5 days later, daily systemic injections of estradiol benzoate (EB) commenced. The females were then brought into sexual receptivity every third day with systemic injections of 500 ug progesterone (P) mixed in propylene glycol, and behavioral tests with sexually vigorous Long Evans males were conducted. During each test, lordosis quotients (LQs) were measured prior to drug administration and hourly for 4 hrs after. The order of drugs tested was varied for each rat. Additionally, the females' weights and gross startle responses were monitored approximately once a week. This procedure enabled us to study the effects of cholinergic antagonists scopolamine methyl bromide (S), atropine sulfate (A) and pirenzepine (P, an M1 blocker); the cholinergic agonist, carbachol (C); the nicotinic ganglionic blocker, hexamethonium (H); and different dilutions of E in cholesterol (1:75, 1:150, 1:250) on the lordotic behavior of exogeneously-primed female rats. The findings were: 1) S inhibited lordosis (L) in 13 rats ($\bar{X}$% inhibition = 71) and had no effect in 7 rats; 2) A inhibited L in 11 rats ($\bar{X}$% inhibition = 91) and had no effect in 1 rat; 3) C facilitated L in all 5 rats tested (maximal facilitation was from a baseline LQ of 0 to 100); 4) P had no effect on L in 5 rats tested; 5) H did not affect L in 4 rats, but had a slight inhibitory effect in 1; 6) 1:75 E + systemic P produced L in 10 out of 13 rats tested ($\bar{X}$LQ = 90); 7) 1:150 E + P produced L in 3 out of 10 rats ($\bar{X}$LQ = 80); 8) 1:250 E + P did not produce lordotic behavior in any of 7 rats tested. Additionally there were no significant changes observed in the weight and startle responses of the female rats. The latency to the onset of behavioral effects after drug application occurred within 1 hr. In 3 rats, the effects of S, A and P were tested in each animal. In 3 other rats, the effects of C, A and S were tested in each animal. Carbachol facilitated L while A and S inhibited it in the same rats. The latencies to the onset of both behavioral effects were similar. (<1hr). These data demonstrate robust cholinergic effects on L in the basomedial hypothalamus of rats.

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378.6 INTRAHIPPOCAMPAL INJECTIONS OF PIRENZEPINE SELECTIVELY IMPAIR REPRESENTATIONAL MEMORY. W.S. Messer, Jr., W.P. Hoss and G.J. Thomas. Center for Brain Research, University of Rochester School of Medicine and Dentistry, Rochester, N.Y. 14642.

Pirenzepine, a novel muscarinic antagonist, distinguishes subtypes of muscarinic receptors within the rat brain on the basis of affinity. High affinity pirenzepine sites (termed $M_1$) are found in highest concentrations in the forebrain, while low affinity sites ($M_2$) predominate in the brainstem. The distribution of pirenzepine binding sites appears quite distinct from the binding profile for muscarinic agonists such as carbamylcholine. The functional significance of the selectivity of pirenzepine for lower affinity agonist sites is not well understood.

In an earlier study (Messer et al., Soc. Neurosci., 1983), we measured the effects of intracranial and intraperitoneal injections of scopolamine (a classical muscarinic antagonist) on the performance of a representational memory task (a kind of alternation). Although systemic injections of scopolamine decreased the percentage of correct responses, running times were also affected and the deficit could not be classified purely as a representational memory impairment. Hippocampal injections of the drug were more effective in selectively producing representational memory deficits, although some animals still showed marked increases in response times, perhaps due to diffusion of the drug to surrounding areas. The present study measures the effects of pirenzepine on the performance of the representational memory task to determine if a ligand with higher selectivity for the hippocampus would be more effective in producing representational memory deficits.

Rats were trained to perform a representational memory task in a T-maze. Repeated exposures to the maze and the food habituated the animals to the test environment. Once the animals demonstrated proficiency in the maze, they were anesthetized and implanted stereotaxically with cannulae aimed at left and right hippocampi. Animals were given one week to recover from surgery before being injected with saline bilaterally to provide a control from which to compare the effects of pirenzepine injections. While injections of saline failed to produce an impairment of performance on the representational memory task, bilateral injections of pirenzepine (0.5 µl of 69.1 mg/ml pirenzepine in saline in each injection) into both hippocampi caused a significant ($p < .001$) reduction in the percentage of correct responses (71%). Rats also were impaired (73%) following an injection of saline on the subsequent day. Running times were not affected on either day, indicating that the deficit was due to an impairment of representational memory processing. Tolerance developed rapidly as shown by the failure of subsequent injections of either pirenzepine or saline to impair performance. The data indicate that pirenzepine acts selectively on hippocampal muscarinic receptors involved in representational memory processing, as was predicted from examination of high affinity binding sites in the rat brain.

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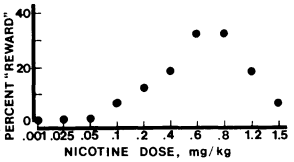
378.7 THE MOUSE PORSOLT BEHAVIORAL DESPAIR TEST: ACTIVITY OF CHOLINERGIC AGONISTS AND ANTAGONISTS. R.E. Chipkin* and B.A. Marasca* (Spon: N. Eisenstein). Schering Corp., 60 Orange St., Bloomfield, NJ 07003.

The Porsolt swim test uses drug-induced decreases in an animal's immobility as a measure of antidepressant (AD) activity and has been proposed as a potential screening procedure for these drugs. In this lab, the test is conducted in the following manner: mice are injected with either vehicle or drug and 60 min later placed in a circular tank (diameter = 14.5 cm) filled with water to a depth of 11 cm. The mouse is permitted to swim for 2 min and then the total time spent immobile during the following 4 min is measured. Typical ADs like desipramine (DMI) and MAO inhibitors reduce immobility times (ITs) in this test relative to vehicle-treated control animals, e.g., the minimal effective dose (MED) to produce a significant ($P<.05$) decrease in ITs for DMI is 30 mg/kg ip and for tranylcypramine is 15 mg/kg ip. However, atypical ADs such as trazodone and mianserin are inactive in this test and at high doses (MED > 10 mg/kg ip) increase ITs. The test might still be useful for screening if it only missed these "true positives". Unfortunately, a variety of agents from diverse pharmacological classes without clinical AD activity can also reduce ITs and as such are false positives. For example, it is well known that muscarinic antagonists are active in this test (atropine's MED = 0.3 mg/kg ip). Unexpectedly, muscarinic agonists such as pilocarpine and oxotremorine are also active (MED = 0.1 and 0.03 mg/kg ip, respectively). Further, both nicotinic agonists (e.g., nicotine) and antagonists (e.g., mecamylamine) reduced ITs (MED = 0.1 and 1 mg/kg ip, respectively) as did cholinesterase inhibitors such as physostigmine (MED = 0.1 mg/kg ip). In general, a screening procedure that fails to identify known true positives has limited utility. Furthermore, a test that consistently targets false positives and cannot discriminate agonists from antagonists within the same class has the additional limitation of requiring multiple secondary tests to separate out drugs with the desired profile. In summary, these results suggest that the Porsolt swim test in mice should only be used as a broad, primary screen and by itself has little predictive power for identifying clinically useful ADs.

378.8 NICOTINE(NIC)-INDUCED CONDITIONED PLACE PREFERENCE (CPP): DOSE-RESPONSE, TEMPORAL REQUIREMENTS AND LACK OF TOLERANCE DEVELOPMENT. P.J. Fudala* and Edgar T. Iwamoto, Department of Pharmacology and Tobacco and Health Research Institute, Univ. of Kentucky, Lexington, KY 40536.

In order to substantiate our previous findings (PBB 22:237, 1985) that NIC causes "reward" and "aversion" in rats, experiments were designed to evaluate the dose-response and time-response of NIC, and the effects of repeated nicotine conditioning and testing in the CPP paradigm.

Methods. On alternate days for 6 da, adult male Sprague-Dawley rats were subcutaneously injected with either saline (SAL, 1 ml/kg) and placed in the preferred compartment, or injected with NIC (0.001-1.5 mg/kg) and placed in the non-preferred compartment of a 3-compartment CPP apparatus. Conditioning sessions were 20 min. On test day, rats ambulated between a central compartment and the two conditioning compartments for 15 min. The durations spent in the saline-paired (S) and drug-paired (D) compartments determined a "residence ratio", RR=(S-D)/(S+D), for each animal. After calculating 1 SD from the control group, drug-paired rats were categorized as exhibiting "reward" if their RR was 2 SD < the mean RR of saline controls. If RR (drug) > [2 SD+$\overline{RR}$ (control)], rats were categorized as exhibiting "aversion". The temporal relationship of NIC-induced CPP was examined by injecting rats with NIC 0.8 mg/kg or SAL at 0, 60 or 120 min prior to conditioning. To ascertain development of tolerance to NIC-induced reward, groups of rats were conditioned with NIC 0.8 mg/kg or SAL over the usual 7 da conditioning and testing period. After 2 da, this sequence was repeated, and after 2 more da, repeated a third time.




Results. Within the dose range of 0.2 to 0.8 mg/kg, NIC reward was positively correlated with dose. In contrast to our previous interpretations, no dose-relation was found for aversion. NIC reward diminished at the 1.2 and 1.5 mg/kg treatment levels. The time-response experiments indicated that animals administered NIC 60 or 120 min prior to conditioning exhibited neither reward nor aversion. The proportion of animals categorized as exhibiting "reward" and "no effect" did not change over the 3 testing sessions: proportion "reward" = 6/16, 6/16 and 9/16; proportion "no effect" = 9/16, 8/16 and 6/16. The data suggest that NIC is rewarding in the CPP paradigm, that the NIC reward cue occurs within 60 min of injection, and that tolerance apparently does not develop to NIC-induced reward. (Supported by the Kentucky Tobacco Research Board).

379.1 INFLUENCE OF $A_1$ NORADRENERGIC REGION STIMULATION ON THE NEURONAL EXCITABILITY OF THE MEDIAL PREOPTIC AREA (mPOA). Y.I. Kim*, C.A. Dudley and R.L. Moss. Dept. of Physiology, Univ. Texas Hlth. Sci. Ctr., Dallas, TX 75235.

Norepinephrine (NE) in mPOA has been implicated in the control of several physiological functions including gonadotropin secretion. One of the major noradrenergic inputs to mPOA has been shown to be the $A_1$ noradrenergic cell group of the caudal medulla. The role of the $A_1$ cell group in controlling the excitability of mPOA and septal neurons was studied with electrophysiological methods in urethane (1.3 mg/kg, IP) anesthetized, ovariectomized female Sasco rats (240-340 g). A multi-barrelled glass microelectrode was used for extracellular recording of single units and iontophoretic application of drugs. Peri-stimulus histograms were generated to evaluate the effect on cell firing of paired cathodal monophasic pulses (50-120μA, 0.1-0.2 msec duration, 20 msec intrapair interval) delivered to the $A_1$ region through a stainless steel bipolar electrode (0.2-1.0 Hz frequency range). For antidromic identification of mPOA-septal cells projecting to median eminence (ME) biphasic pulses (1-2 mA, 0.2 msec duration) were applied to ME. At the end of each experiment histological verification of stimulating and recording sites was performed.

Of 71 cells recorded, stimulation of the $A_1$ region evoked orthodromic responses from 22 cells. Nineteen cells (86%) were excited whereas 3 cells (14%) were inhibited. These 3 inhibited cells were not further analyzed. Of the 19 excited cells one was activated antidromically from ME stimulation. Excited cells displayed a short latency (46.2±8.7 msec, mean±S.E.M.) and a relatively long duration ($\bar{x}$=300.7±54.0 msec) response. Effects of sotalol (β-noradrenergic antagonist) on these stimulus-bound excitations were assessed in 13 excited cells by applying the drug iontophoretically during generation of a "sotalol" histogram. The ratio between firing rates during the response epoch and during the period of spontaneous discharge was calculated from the "sotalol" histogram and compared to that of its control histogram. Sotalol decreased the ratio in 7 cells and increased it in 1 cell. Iontophoresed NE effects on spontaneous firing rate were also tested in 6 of these excited cells and, additionally, in 5 cells unresponsive to $A_1$ region stimulation. In the excited cell group, spontaneous firing rate was affected by NE in two neurons (one increased, one decreased), whereas in the unresponsive cell group, only one neuron was affected (decreased).

The results suggest that the predominant effect of $A_1$ noradrenergic input on the mPOA-septal cells is excitatory, that mPOA-septal cell(s) projecting to ME can be influenced by $A_1$ region stimulation, and that sotalol may block the stimulus-bound response by acting on the noradrenergic receptors. Studies involving α-noradrenergic antagonist are presently in progress to further characterize the noradrenergic receptors. Supported by NIH grant NS-10434.

379.2 Effects of Ventral Medial Preoptic Nucleus (vMPN) and Suprachiasmatic Nucleus (SCN) Lesions on the LHRH Neuronal System of the Female Rat. O.K. Ronnekleiv, M.J. Kelly and B.R. Naylor*, Dept. of Physiology, Oregon Health Sciences University and Oregon Regional Primate Research Center, Portland, OR 97201.

Lesions of the SCN or vMPN disrupt the estrous cycle of the female rat and prevent the preovulatory surge of LH and PRL. It is not known whether these lesions affect both the synthesis and/or release of brain peptides (e.g. LHRH) that control the preovulatory surge of the pituitary hormones. We designed the present study to determine the effects of discrete lesions of the SCN or vMPN on LHRH neurons of the female rat. We made bilateral electrolytic lesions by passing 1-5 μA current for 3 min. through Wood's metal electrodes (70μm tip; 2-10 MΩ). Vaginal cytology and hormonal profiles of LH, PRL, estrogen and progesterone were assessed for each individual animal (see Dykshoorn et al., Soc. Neurosci. Abstr., Vol. XI, 1985). Two to three months following the lesions, control (N = 8) and lesioned-animals (N = 8) were perfused intracardially with 500-600 ml of 4% paraformaldehyde in phosphate buffer. We cut the brain into 50 μm coronal sections on a vibratome and processed the tissue for LHRH-immunocytochemical staining using the PAP method and a high titer, conformational antiserum (EL-14) which recognizes the mature decapeptide (Ellinwood et al., Peptides 6:45-52, 1985). The number of LHRH neurons between the septal area (Konig and Klippel:A9410) and caudal medial basal hypothalamus (MBH; K&K:A3750) were counted in alternate sections and extrapolated to the total number of neurons for all sections. Other sections were used for immunocytochemical controls. Faintly stained LHRH neurons were observed in both the POA and MBH in all animals at all stages of the cycle. The number of immunoreactive cell bodies varied from a high of 595 in animals sacrificed on the morning of proestrus, to a low of 41 in animals sacrificed on estrus ($\bar{x}$ = 279 ± 84; N = 8). In contrast, the constant estrous animals with lesions showed an increased density of LHRH neurons rostral, lateral, and caudal to the lesion. Qualitatively, the intensity of staining was more dense in neurons throughout the POA-MBH, and quantitatively the numbers ranged from 575 to 980 cells per animal ($\bar{x}$ = 779 ± 41, N = 8; $p<.001$ vs. control). Moreover, all lesioned animals exhibited intense fiber stain in the median eminence region. Together, these data indicate that the lesion-induced constant estrous female rat is capable of synthesizing LHRH, but the neural signal for its release is disrupted. (Supported by PHS Grants HD 16793, HD 19905 RR00163.)

379.3 STIMULATORY EFFECT OF GABA AND A GABA AGONIST ON LUTEINIZING HORMONE-RELEASING HORMONE (LHRH) RELEASE FROM HYPOTHALAMIC FRAGMENTS IN VITRO. C. Masotto* and A. Negro-Vilar (SPON: N. Kreisman). Reproductive Neuroendocrinology Section, Lab. Reprod. Dev. Tox., NIEHS, NIH, Research Triangle Park, NC 27709.

The neurotransmitter γ-aminobutyric acid (GABA) is found in high concentration in the hypothalamus and has been reported to be involved in the control of anterior pituitary hormone secretion. The role of GABA and its analogs in the regulation of gonadotropin secretion has not yet been fully clarified, with some studies suggesting a stimulatory effect of GABA on LH secretion, while others favoring an inhibitory role. In terms of the site of action, however, the scant evidence available seems to indicate a central (hypothalamic) site. In order to explore in more detail these issues, we performed experiments to evaluate the direct effect of GABA and its specific agonist, muscimol, on LHRH release in vitro. Adult intact male Sprague-Dawley rats served as tissue donors. A hypothalamic fragment consisting of the intact arcuate nucleus-median eminence region (ARC-ME) and surrounding structures was dissected and incubated in vitro in a Krebs buffer. LHRH release was evaluated during a 30-minute incubation period following a 30-minute preincubation. GABA and muscimol at the concentration of $10^{-4}$M significantly increased LHRH release. Different concentrations of muscimol ($10^{-6}$ to $10^{-4}$M) produced graded increases in LHRH release. In addition, the effects of muscimol were completely blocked in the presence of the specific GABA receptor antagonist, bicuculline. Addition of bicuculline to the incubates in the absence of the agonist did not affect basal release of LHRH. These results provide evidence supporting a stimulatory role of GABA on LHRH release and, indirectly on gonadotropin secretion. Further, the results also suggest that GABA-ergic receptors located within the arcuate-median eminence region are stimulatory to LHRH release. Since GABA is in general an inhibitory neurotransmitter, the data may indicate that its effects are possibly mediated by interacting with another inhibitory neuron located within this region. Recent studies from our laboratory indicate that blockade of opiate receptors in ARC-ME fragments in vitro results in a stimulation of LHRH release. Therefore, we hypothesize that GABA may interact with this or other inhibitory systems within this region to modulate LHRH secretion.

379.4 GNRH SECRETION IN VITRO: INTERACTIONS BETWEEN ESTROGEN AND NOREPINEPHRINE DURING DEVELOPMENT. R.W. Clough, C.D. Sladek and G.E. Hoffman. Dept. of Anatomy and Neurology, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642.

Evidence from both in vivo and in vitro studies suggest that secretion of gonadotropin releasing hormone (GNRH) is controlled by a catecholaminergic system. It is also recognized that estrogen plays a major role in control of anterior pituitary gland gonadotropin secretion in females. In addition, catecholamine neurons have been found to sequester tritiated estrodiol while GNRH neurons have not. Our present studies are designed to investigate the complex interactions between estrogen, catecholamines and GNRH release from preoptic area-mediobasal-median eminence (POA-MBH-ME) explants in an in vitro perifusion system. Series one: Adult female rats were ovariectomized (ovx'ed) two weeks prior to receiving a subcutaneous injection of estradiol benzoate (EB, 10 ug/rat) or an oil vehicle. At 1100 hours, two days following EB, rats were decapitated, brains were excised and the POA-MBH-ME explants were placed into individual 500 ul incubation chambers. Six microchambers were perifused simulataneously with tissue culture media (Gibco, F12) at a flow rate of 20 ul/minute. Effluents of the chambers were collected in 15 minute fractions and GNRH concentration was measured by specific radioimmunoassay. The POA-MBH-ME explants were exposed to norepinephrine (NE) pulses of known concentration and duration. Near completion of each series, the explants were perifused with a pulse of 57 mM potassium chloride to assess their viability. NE induced a significant increase in GNRH release from explants taken from ovx'ed animals both with and without EB replacement ($p$ .05). However, following termination of the NE pulse, the oil treated group did not return to the prestimulus baseline while the EB treated explants showed an immediate return to baseline. Series two: Prepuberal female rats were administered EB or oil vehicle on day 25 after birth in order to induce a proestrus-like gonadotropin surge on the afternoon of day 27. Prior to the expected gonadotropin surge, the POA-MBH-ME explants were excised as previously and placed into the multiple microchamber perifusion system described. NE was pulsed over the explants as in the previous series of experiments. In this series, NE induced a significant elevation in GNRH release from explants of EB-treated prepuberal females but had no effect on GNRH release from the non-EB treated prepuberal rat explants. These studies indicate that catecholamine mediated GNRH secretion is activated by prior exposure to estrogen; however, continued exposure to estrogen may not be required to maintain at least the stimulatory effect of NE on GNRH release. This interaction between EB, NE and GNRH release may be one of the mechanisms underlying the estrogen dependence of puberal activation of phasic pituitary gland gonadotropin secretion in females.

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379.5 HYPERPROLACTINEMIA ATTENUATES OVARIAN STEROID INDUCED INCREASES IN SERUM LH LEVELS AND REGION-SPECIFIC SEROTONIN SYNTHESIS IN OVARIECTOMIZED RATS. ARE THESE EFFECTS RELATED? T. S. King, A. J. Carrillo and W. W. Morgan. Dept. Cellular Structural Biology, Univ. Texas Hlth. Sci. Ctr., San Antonio, TX 78284.

Hyperprolactinemia adversely affects reproductive function, presumably through an effect at the hypothalamic level (del Pozo et al., Obstet. Gynecol. 46: 539, 1975). Given the numerous published reports linking hypothalamic serotonin (5-hydroxytryptamine: 5HT) mechanisms to the regulation of gonadotrophin secretion (see: Kalra and Kalra, Endocr. Rev. 4:311, 1983), we sought to determine the effects of experimentally-induced hyperprolactinemia on ovarian steroid-induced increases in serum LH levels and region-specific hypothalamic 5HT synthesis in ovariectomized rats. In the first study, bilaterally ovariectomized Sprague-Dawley rats either received two pituitary homografts implanted beneath the left kidney capsule or were sham-grafted. Both groups of rats were injected s.c. with 5µg · $100g^{-1}$ of estradiol benzoate ($E_2$) in corn oil vehicle at 0800h, one and two days before serum collection and 5 $mg \cdot 100g^{-1}$ of progesterone (P) in corn oil vehicle at 0700h on the day of serum collection. Blood samples were collected via chronic jugular cannula from each rat at 1000h, 1200h, 1300h, 1400h, 1600h and 1800h. A statistically significant elevation in serum LH levels was detected at 1300h, 1400h and 1600h. This increase in serum LH levels was significantly attenuated in rats bearing pituitary-homografts, an effect attributed to the high serum PRL levels measured in these animals. In the second study, bilaterally ovariectomized Sprague-Dawley rats were divided into three experimental groups: (1) rats bearing two pituitary homografts and injected with $E_2$ and P on the schedule and at the dosages previously described, (2) sham-grafted rats injected with $E_2$ and P on the schedule and at the dosages previously described and (3) sham-grafted rats injected with corn oil vehicle only. The rats were then injected i.v. with 100 $mg \cdot kg^{-1}$ of NSD-1015 six hours after P injection and 30 min prior to killing the rats. Accumulation of 5-hydroxytryptophan in various hypothalamic areas was then assayed by LCED as a relative index for the level of 5HT synthesis. Administration of these ovarian steroids produced an increase in estimated 5HT synthesis in the preoptic area-anterior hypothalamus (POA-AH) but not in the median eminence or mediobasal hypothalamus. The $E_2$ + P induced increase in 5HT synthesis in the POA-AH was significantly blunted in rats with pituitary homografts. Considering the postulated role a 5HT mechanism within the POA-AH plays to facilitate ovarian steroid-mediated surges in LH secretion, hyperprolactinemia may inhibit LH secretion through its attenuation of ovarian steroid stimulation of 5HT synthesis in the POA-AH. (Supported by NIH grant RR07187 and NIH grant HD10292 [Neuroendocrine Core]).

379.6 ROLE OF CATECHOLAMINES AND OPIATES IN STIMULATION OF LH RELEASE BY NEUROPEPTIDE Y (NPY). L.G. Allen*, W.R. Crowley* and S.P. Kalra, (SPON. B. Cooper) Dept. OB-Gyn, Univ. Fla. Col. Med., Gainesville, FL 32610 and Dept. of Pharmacol., Univ. Tenn. Col. Med., Memphis, TN 38163

We have shown that intraventricular NPY (0.5-10 µg/rat) stimulated LH release in estradiol benzoate, progesterone (EBP)-primed ovariectomized rats in a manner similar to that reported for norepinephrine (NE) and epinephrine (E). Because of the neuronal coexistence of NE, E and NPY and the close anatomical relationships between NPY, opioids and LHRH neurons in the hypothalamus, we investigated the involvement of catecholamines and opiates in the NPY-induced LH response. Twelve days after ovariectomy and placement of permanent cannulae in the third ventricles, rats received EB (30 µg/rat) and P (15 mg/rat). Two days later, catecholamine receptor antagonists or synthesis inhibitors were administered prior to Ivt NPY injection. Blood samples were withdrawn through indwelling atrial catheters prior to and at 10, 20, 30 and 60 min after Ivt NPY. NPY (2 µg, previously shown to evoke maximal LH response) significantly stimulated LH release in the EBP rats; peak levels at 10 min and significantly high LH levels were evident at 20 and 30 min. $\alpha_1$-adrenergic receptor antagonist, prazosin (1 or 3 mg/kg), ß-adrenergic receptor antagonist, propranolol (10 mg/kg) or dopamine receptor antagonist, pimozide (0.63 mg/kg) failed to block the NPY-induced stimulation of LH release. In fact, the high dose of prazosin potentiated the NPY-induced LH response. Rats pretreated with phentolamine (10 mg/kg) failed to show LH stimulation after NPY administration, but a subsequent study revealed that phentolamine on its own stimulated LH release. Prior suppression of hypothalamic E stores with a PNMT inhibitor LY 78335, 2,3-dichloro-methyl-α-benzylamine (50 mg/kg), delayed the NPY-induced LH response; a significant LH rise was evident only at 20 min. However, suppression of NE and E levels by a DBH inhibitor, diethyldithiocarbamate (550 mg/kg), suppressed LH release elicited by NPY. Stimulation of opiate receptors with morphine (20 mg/kg, s.c.) failed to block the NPY-induced LH response. These studies show that stimulation of LH release by NPY may occur independently of postsynaptic adrenergic or opiate receptor mediation. However, the findings that NPY stimulation of LH release was delayed or interrupted by catecholamine synthesis inhibitors, suggest that a complex interaction between hypothalamic NPY and adrenergic neurons may be involved in the regulation of LH release. (Supported by NIH HD 08634).

379.7 ROLE OF CENTRAL SEROTONIN SYSTEMS IN THE STIMULATORY EFFECTS OF OVARIAN HORMONES AND NALOXONE ON LH RELEASE IN FEMALE RATS. M.D. Johnson* and W.R. Crowley. Dept. of Pharmacology, University of Tennessee, Memphis, TN 38163

The present experiments tested whether serotonergic neurons that innervate discrete areas of the hypothalamus are involved in the stimulation of LH release by ovarian hormones or by the opiate antagonist, naloxone. Two weeks after ovariectomy, rats received either oil vehicle or estradiol benzoate (EB). Forty eight hours later, one group of EB-treated rats was decapitated; the remainder received either oil vehicle or progesterone (P) and were decapitated three or six hours later. Thirty minutes prior to sacrifice, half the animals in each hormone treatment group received saline, while the other half received the monoamine oxidase inhibitor, pargyline HCl. LH was measured in serum by radioimmunoassay. Discrete hypothalamic nuclei were removed by the microdissection technique, and serotonin (5-HT) was measured by liquid chromatography with electrochemical detection. Two days after EB, LH concentrations were reduced in the morning, but rose by late afternoon. Administration of P to EB-primed rats stimulated an afternoon LH surge. EB plus P treatment also enhanced the pargyline-induced accumulation of 5-HT, suggesting increased 5-HT activity, in the medial preoptic nucleus and interstitial nucleus of the stria terminalis. EB plus P treatment decreased 5-HT accumulation in the ventromedial nucleus, suggesting decreased serotonergic activity. 5-HT levels or accumulation after pargyline were unchanged after EB alone. Ovarian hormones did not affect 5-HT in the arcuate - median eminence region. The administration of naloxone (10 mg/kg) to EB-primed rats also stimulated LH release one hour later, but did not affect 5-HT concentrations or the pargyline-induced accumulation of 5-HT in any of the nuclei dissected. To test the relationship of the ovarian hormone-induced changes in 5-HT activity to the stimulation of LH release, the indoleamine neurotoxin, 5,7-dihydroxytryptamine (5,7 DHT) was microinjected into the preoptic area to deplete 5-HT locally. Such treatment blocked the LH surge induced by EB plus P, in association with decreased 5-HT and unchanged catecholamine levels in the preoptic area.

The present results suggest that 1) central 5-HT neurons innervating the preoptic area are important for the estradiol/progesterone-induced LH surge, 2) changes in 5-HT activity may not mediate the inhibitory or stimulatory effects of estradiol alone on LH release, and 3) endogenous opioids may not affect LH secretion via an interaction with central 5-HT systems.

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379.8 CHRONIC MORPHINE TREATMENT ENHANCES THE POSITIVE AND NEGATIVE FEEDBACK EFFECTS OF ESTRADIOL ON GONADOTROPIN SECRETION IN OVARIECTOMIZED RATS. S.M. Gabriel, L.A. Berglund* and J.W. Simpkins, Dept. of Pharmacodynamics, College of Pharmacy, University of Florida, Gainseville, FL 32610.

The influence of endogenous opioid containing neurons on the feedback regulation of gonadotropin secretion was evaluated using female rats. Rats, ovariectomized for 2 weeks, were chronically exposed to morphine (75 mg freebase, s.c. implants, plus 2 additional implants every 2 days) or placebo treatments. After 2 days of morphine exposure, rats were given silastic implants containing 17-ß-estradiol (E2). The silastic implants were 10 mm E2, 5 mm E2 or 5 mm tubes filled with E2 which had been first diluted in half with cholesterol. Serum LH concentrations were determined in blood samples taken in the morning (1000h) and afternoon (1600h) at 2 days after the E2 treatment. E2 implants alone caused a dose-dependent decline in serum LH concentrations at 1000h and each dose caused a midafternoon surge in LH. Chronic morphine treatment alone was ineffective in altering morning levels of LH but it further reduced morning levels of LH when combined with each of the 3 doses of E2. Similarly the midafternoon surge in LH was enhanced by the combination of morphine plus each dose of E2. A detailed time course revealed that chronic morphine exposure advanced the time of onset and increased the magnitude of the E2-induced LH surge. After 4 days of exposure to E2, chronic morphine treatment continued to enhance the inhibitory, but not the stimulatory, effects of E2 on LH secretion. Similar, albeit less pronounced, effects of chronic morphine treatment on the inhibitory and stimulatory action of E2 on FSH secretion were observed. These results indicate that morphine enhances both the negative and positive feedback effects of E2 on gonadotropin secretion in the female rat and suggest that endogenous opioid peptides may play a role in altering the set point of the brain gonadostat. Supported by NIH grants AG02021 and HD14075 to JWS and AM7561 to SMG.

379.9 A SEX DIFFERENCE IN NEUROENDOCRINE MECHANISMS REGULATING THE ACUTE PITUITARY FOLLICLE-STIMULATING HORMONE RESPONSE TO CASTRATION. P.V. Berardo* and L.V. De Paolo* (SPON:A. Carrillo). Dept. of Physiol., Univ. Tx. Hlth. Sci. Ctr., San Antonio, TX 78284.

It is well known that acute pituitary gonadotropin responses to castration differ between male and female rats. Whereas increases in plasma levels of LH and FSH levels occur within 12h after orchidectomy (ORDX), only FSH levels are increased immediately after ovariectomy (OVX). Therefore, the following experiment was conducted to ascertain the role of the central nervous system in mediating pituitary FSH responses to ORDX and OVX in hopes of identifying a possible sex difference in central mechanisms controlling these responses. Adult male rats and 4-day cycling female rats on diestrus-1 were injected i.p. with either phenobarbital sodium (Pb, 80 mg/kg BW) or vehicle at 0800h. Following a blood collection at 1000h, rats given barbiturate or vehicle were either sham-castrated or castrated under ether. Additional blood samples were obtained at 3,8,13,18 and 24h after castration. Supplemental injections of Pb or vehicle were given at these times. Plasma levels of LH and FSH were measured by RIA. Compared to sham-castrated rats, plasma LH and FSH levels were elevated by 13h after ORDX. Increases in plasma levels of both gonadotropins after ORDX were completely prevented by Pb. In female rats, initial increments in plasma FSH levels occurred between 3 and 8h after OVX. However, in contrast to ORDX, Pb treatment did not prevent initial rises in plasma FSH levels at 8h after OVX and only partially suppressed elevated OVX-induced increases in plasma FSH levels between 13 and 24h. Plasma LH levels were not elevated by 24h after OVX.

In order to specifically evaluate the role of luteinizing hormone-releasing hormone (LHRH) in mediating the Pb-sensitive rises in gonadotropins after castration, groups of male rats and female rats on estrus were injected s.c. with 400 µg of a potent LHRH antagonist (ALHRH) or oil at 1200h. At 1000h on the next morning, an initial blood sample was taken and all rats were castrated under ether. Additional blood samples were taken as described in the previous experiment. Similar to Pb, ALHRH completely abolished ORDX-induced increases in circulating LH and FSH levels. In contrast to Pb, ALHRH partially suppressed increases in plasma FSH levels 8h after OVX, but like Pb, ALHRH partially suppressed FSH levels between 13 and 24h. The difference in results obtained in female rats using Pb vs. ALHRH may be attributed to an incomplete suppression of LHRH release by Pb.

These results clearly demonstrate that acute increases in plasma LH and FSH levels after ORDX are totally mediated by LHRH. In contrast, acute increases in plasma FSH levels after OVX are due to both an LHRH-dependent and LHRH-independent mechanism (i.e. increase in basal FSH secretion). (Supported by NIH grant HD-17807).

379.10 PHASE SPECIFIC STARVATION DURING THE ESTROUS CYCLE BLOCKS HAMSTER OVULATION. L.P. Morin. Dept. Psychiatry, SUNY, Stony Brook, NY 11794.

Reproductive capacity of female hamsters, as estimated by the ovulatory response, is particularly susceptible to interference by food deprivation. Previous studies (Printz & Greenwald, Neuroendocrinology 7:171, 1971; Morin, Biol. Reprod. 13:99, 1975) showed that hamsters generally fail to ovulate if deprived of food for one or two estrous cycles. The present studies demonstrate that starvation which is specific to the two days immediately following ovulation will block the next expected ovulation in about 80% of the animals.

When placed with vigorous males, food deprived animals which subsequently failed to ovulate also failed to show lordosis behavior at the expected estrous cycle time. With exogenous estradiol benzoate provided, animals showed short latency lordosis despite food deprivation. This suggests that endogenous estradiol levels are insufficient for behavior change. Starvation also resulted in significantly smaller ovarian follicle sizes. The preovulatory gonadotropin release pattern was altered by phasic food deprivation with the afternoon proestrous surge of LH apparently eliminated. FSH levels on the morning of proestrous were elevated compared to control levels. Efforts to facilitate ovulation with prolactin or gonadotropins given at several different estrous cycle times were not successful.

The results suggested that phasic food deprivation blocks a rather specific set of neuroendocrine events which retard follicular development. As a consequence, estradiol release is inadequate to prime the neuro-endocrine system for lordosis behavior and the ovulatory release of LH.

379.11 EVIDENCE FOR ALPHA$_2$ ADRENERGIC MEDIATION OF THE STIMULATORY EFFECTS OF ANGIOTENSIN II (AII) AND NOREPINEPHRINE (NE) ON LUTIENIZING HORMONE (LH) RELEASE IN FEMALE RATS. M.K. Steele and W.F. Ganong, Department of Physiology, University of California, San Francisco, 94143.

We have hypothesized that intraventricular (IVT) infusion of AII affects LH secretion by altering brain NE release (Steele, et al, Neuroendocrinology 40:210, 1985). We have tested this hypothesis by observing the effects of AII on LH release in animals treated with either the alpha$_1$ adrenergic antagonist, prazosin (1.0 mg/kg, i.p.) or the alpha$_2$ adrenergic blocker, yohimbine (3.5 mg/kg, i.p.). Animals infused IVT with NE were also tested.

Ovariectomized rats, implanted with a cannula aimed at the third cerebral ventricle, were treated s.c. with estradiol benzoate (50 ug) and progesterone (25 mg) 72 h prior to use in experiments. A jugular cannula was inserted 24 h prior to blood collection. On the day of the experiment, a "basal" blood sample (100 µl whole blood) was withdrawn, followed immediately by injection of prazosin, yohimbine or saline vehicle. Continuous whole blood samples (100 µl/15 min) were withdrawn via peristaltic pumps for 1.5 h, at which time a 30 min infusion of artificial cerebrospinal fluid (aCSF, 25 µl/h), AII (600 ng/25 µl/h) or NE (15 ug free base/ 25 µl/h) was begun. Blood withdrawal continued during and for 1.0 h following the IVT infusion. Whole blood concentrations of LH were determined by RIA. Water intake was also monitored in these experiments.

Both prazosin and yohimbine produced moderate increases in blood LH levels at 30 and 60 min, which returned to baseline by the start of the IVT infusions. Prazosin administration did not affect either the AII- or NE-induced increase in blood LH levels. Yohimbine treatment, on the other hand, prevented the rise in blood LH produced by AII and by NE infusion.

Infusion of AII increased water intake compared to aCSF or NE administration. Neither prazosin nor yohimbine treatment modified water ingestion due to AII. During the 1.5h preinfusion time period, yohimbine alone increased water intake compared to treatment with prazosin alone.

These data suggest that the stimulation of LH release induced by AII and by NE in steroid-treated female rats is mediated by an alpha$_2$ adrenergic mechanism.

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379.12 EVIDENCE THAT VASOACTIVE INTESTINAL PEPTIDE IS SYNTHESIZED IN ANTERIOR PITUITARY TISSUE. M.A. Arnaout,* D.R. Martinson,* T.C. Hagen,* T.L. Garthwaite. VA Medical Center and the Medical College of Wisconsin, Milwaukee, WI 53193.

Vasoactive intestinal peptide (VIP) is a putative hypothalamic prolactin (PRL) releasing factor based on its presence in the hypothalamus, its release into hypophysial portal blood, and its ability to release prolactin from anterior pituitary tissue in vitro. Although several studies have demonstrated that VIP is also present in the anterior pituitary, the origin and functional role of pituitary VIP are unknown. Our observation that anti-VIP antiserum attenuates the basal release of PRL from cultured anterior pituitary cells suggests that VIP may be synthesized in the pituitary and may exert local effects on PRL release. We report here evidence for intrapituitary synthesis of VIP.

Quartered anterior pituitaries from male rats were washed with Medium 199 and incubated in leucine-free Eagle's media containing 14 µCi/ml $^3$H-leucine for 2 hours. These labeled pituitaries were studied further by a combination of gel chromatography, immunoprecipitation with anti-VIP antiserum, or perifusion.

The incorporation of the $^3$H-leucine label into cell proteins was assessed using Sephadex G-50F gel exclusion chromatography of pituitary homogenates. The majority of the label was found in or near the void volume fractions with a smaller peak of activity coeluting with synthetic porcine VIP. Immunoprecipitation of labeled pituitary homogenates revealed significantly ($p < 0.025$) greater precipitation of the label with anti-VIP antiserum than with non-immune rabbit serum.

Incorporation of $^3$H-leucine into releasable products was assessed by perifusion of labeled pituitaries for 2 hours with Medium 199 at a rate of 2 ml/15 min. followed by a 1 hour perifusion with Medium 199 containing 56 mM KCl. Immunoprecipitation of the KCl-stimulated perifusate fractions obtained significantly greater radioactivity with anti-VIP than with non-immune rabbit serum ($p < 0.025$). Gel chromatography of the KCl-stimulated perifusate revealed two peaks of activity. One peak coeluted with synthetic VIP and contained VIP immunoreactivity by RIA.

Conclusions: VIP appears to be synthesized in anterior pituitary tissue as suggested by a) coelution of labeled peptides from pituitary homogenates and KCl-stimulated perifusates with synthetic VIP, b) immunoprecipitation of labeled peptides from homogenates and perifusates by specific anti-VIP antisera, and c) release of immunoassayable VIP in response to KCl-induced depolarization.

379.13 HYPOTHALAMIC PROLACTIN- AND TYROSINE HYDROXYLASE-IMMUNOREACTIVE NEURONS IN PITUITARY PROLACTIN-DEFICIENT DWARF MICE. C.J. Phelps. Department of Anatomy, University of Rochester Medical Center, Rochester, New York 14642.

Prolactin (PRL)-immunoreactive cells have been described in the hypothalamus of rats (Toubeau et al., J. Endocrinol. 83:261, 1979): these neurons have extensive projections both rostrally and caudally (Shivers et al., SN Abs. 9:108, 1983). CNS production of prolactin implies a genetic question regarding differentiation of pituitary and brain hormone expression. Snell dwarf mice are hypopituitary homozygous recessive mutants which neither translate nor transcribe pituitary PRL, although the PRL gene is intact (Slabaugh et al., Mol. Cell. Endocrinol. 28:289, 1982). Tuberoinfundibular dopamine (DA) is also severely deficient (Morgan et al., Endocrinol 109:2069, 1981; Phelps et al., Cell Tiss. Res., 1985). The present study was undertaken to determine whether hypothalamic PRL or tyrosine hydroxylase (TH) immunoreactivity exist in dwarf (dw/dw) and normal (DW/?) mice of the same strain (DW/J). Brains of young adult mice were processed by intracerebroventricular colchicine pretreatment and vascular perfusion with Zamboni's fixative. Coronal sections (30 um) were processed for PRL immunocytochemistry (ICC) using NIADDK anti-mouse PRL, 1:2000. Specific staining was abrogated by 1) substitution of non-immune serum for PRL antiserum, and 2) pre-absorption of the antiserum with 1.0, but not 0.1, $\mu$M mouse PRL (AFP-4111-E). The PRL antiserum has <1% crossreactivity with other mouse pituitary hormones (A.F. Parlow). TH ICC was performed on adjacent sections, using Eugenetech anti-TH serum, 1:3000.

In the brains of DW/? and dw/dw littermates, PRL-immunoreactive fine-sized varicose fibres were particularly numerous throughout periventricular regions, reached rostrally to medial basal olfactory cortex and to lateral septum, ramified through the supraoptic commissure, to suprachiasmatic and supraoptic nuclei, and were extensive in paraventricular nuclei and in medial basal hypothalamus, extending to internal median eminence and proximal pituitary stalk. No differences in fibre pattern were observed between sexes or between DW/? and dw/dw mice. PRL-immunoreactive perikarya (up to 60 per section) were identified in hypothalamus, primarily in, and extending laterally from, ventral arcuate nucleus, but occasionally in PVN and SON. Cell bodies were less numerous and faintly stained in dwarfs, perhaps due to difficulty in effective colchicine treatment. TH immunoreactivity in cells and fibres of dwarf brains was similar to that in normal mice, except in the tuberoinfundibular DA (A12) region: fewer perikarya were stained, and axons at the ventrolateral border of the arcuate nucleus appeared tortuous and swollen.

These observations indicate that the PRL deficiency in Snell dwarf mouse pituitary does not affect CNS PRL expression, but that the morphology, as well as transmitter content, of tuberoinfundibular DA neurons is adversely affected in the condition.

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379.14 EFFECT OF CYSTEAMINE (CSH) ON SUCKLING-INDUCED PROLACTIN (PRL) SECRETION IN THE RAT. Peter N. Riskind, William J. Millard, and Joseph B. Martin, Department of Neurology, Mass Gen Hosp, Boston, MA 02114.

We have previously reported that CSH administration severely attenuates basal and pharmacologically-induced PRL secretion in the rat. The present experiments investigated the effect of CSH on suckling-induced PRL release, to determine whether CSH also blocks physiologically-induced PRL secretion. Sprague-Dawley rats were implanted with Silastic right atrial catheters on days 14-17 of pregnancy. On the second day postpartum the mothers and their pups (6 pups/litter) were transferred to isolation/blood sampling cages. Blood sampling from the freely-moving unanesthetized rats was done as previously described (Endocrinology 114:1232, 1984) between days 13-16 postpartum. Suckling activity was confirmed by observation of the animals through a small viewing window. The first experiment determined the dose-response of CSH on suckling-induced PRL release. On the day of experimentation pups were removed from their mothers at 0:800 h (lights on from 0:800-20:00 h). At 10:00 h mothers were administered either CSH at doses of 30, 90, or 300 mg/kg, sc or ethanolamine (300 mg/kg, sc) as a control injection. Blood sampling was initiated at 11:30 h with samples taken every 15 min throughout the study. The pups were returned to their mothers at 12:00 h and sampling was continued for another 2 h. In both control animals and those receiving 30 mg/kg CSH, suckling caused a rapid elevation of plasma PRL with peak responses occurring at 60-75 min after returning the pups. No elevation of plasma PRL was found in animals which were administered either the 90 or 300 mg/kg doses of CSH, despite normal nursing behavior by the CSH-treated rats. Moreover, CSH did not acutely affect the amount of milk obtained by the pups, as determined by their weights before and after suckling. The second experiment investigated whether prior suckling, which reportedly causes "depletion-transformation" of pituitary PRL stores, prevents a subsequent inhibitory effect of CSH. Pups were removed from their mothers at 08:30 h, and returned for a 30-min suckling period beginning at 14:30. CSH 90 mg/kg, sc, was given immediately after the pups were removed, and 60 min later the pups were returned for a second 90-min suckling period. Despite previous suckling stimulation, CSH completely inhibited the PRL rise during the second nursing period. These results indicate that CSH administration inhibits physiologically-induced PRL secretion with similar potency and efficacy as previously reported for CSH effects on basal and drug-induced PRL secretion. Furthermore, the effect of CSH is not obviated by a previous suckling stimulus, suggesting that "depletion-transformation" (which presumably occurred) does not protect against the effect of CSH. Supported by grants HD-17364 and AM-32711.

379.15 INTRAVENTRICULAR CHOLECYSTOKININ OCTAPEPTIDE ELEVATES PLASMA PROLACTIN K. Tanimoto*, C.A. Tamminga, M. Knight and T.N. Chase, Experimental Therapeutics Branch, NINCDS, NIH, Bethesda, MD 20205 and Maryland Psychiatric Research Center, Baltimore, MD 21228

Cholecystokinin is found throughout brain, in the hypothalamus and brain stem as well as cortex and basal ganglia. Although its precise physiologic functions remain elusive, many central actions of the peptide have been elucidated. Because CCK-8 is colocalized with dopamine (DA) in certain mesencephalic neurons, the role this peptide might play in CNS DA-modulated functions like prolactin (PRL) secretion is of interest. Prolactin (PRL) release is regulated by a hypothalamic releasing factor and an inhibiting factor. Much evidence indicates that the PRL-inhibiting factor is dopamine. Although the identity of the PRL-releasing factor is uncertain, VIP and PHI are currently thought to be candidates. Therefore, we investigated the effect of CCK-8 on plasma PRL levels in freely moving rats which were implanted with chronic indwelling right atrial catheters.

Intravenous (iv) injections of CCK-8 (4 and 40 ng/rat, and 1 ug/rat) in the chronically cannulated rats did not alter plasma PRL levels. However, plasma PRL levels were significantly elevated following intracerebroventricular (icv) injection of CCK-8 @ 40 ng/rat and 400 ng/rat. Proglumide (0.2 mg/kg,iv) and benzotript (0.2 mg/kg,iv), specific antagonists of cholecystokinin, completely blocked the PRL release induced by CCK-8 (40 ng, icv). The CCK-8 induced rise in PRL was not apparent with haloperidol (0.2 mg/kg,iv), sulpiride (0.1 mg/kg, iv), domperidone (0.1 mg/kg,iv) or RO 22-1319 (0.1 mg/kg,iv). However, the inhibition of PRL secretion by apomorphine (0.1 mg/kg,iv) was significantly antagonized by CCK-8 (40 ng and 400 ng, icv). These results indicate that icv, but not intravenous administration of CCK-8 modifies PRL levels in plasma through its action on a central receptor, possibly by modifying the activity of hypothalamic PRL-releasing and/or inhibiting factor. Our data lead us to speculate that CCK-8 alters plasma PRL levels at a cholecystokinin receptor in the hypothalamus which regulates the PRL-releasing factor.

Plasma PRL levels (ng/ml)

| (min) | (0) | (5) | (15) | (30) |
|---|---|---|---|---|
| Saline | 16.6 ± 2.7 | 15.8 ± 1.3 | 14.6 ± 1.3 | 13.7 ± 1.1 |
| CCK-8(icv) | | | | |
| 4 ng | 18.7 ± 4.2 | 16.8 ± 1.5 | 15.1 ± 2.7 | 12.6 ± 3.1 |
| 40 ng | 16.8 ± 0.8 | 33.2 ± 2.7* | 22.2 ± 1.8* | 15.1 ± 0.4 |
| 400 ng | 15.0 ± 0.4 | 47.0 ± 6.6* | 30.4 ± 6.3** | 21.0 ± 3.7 |

379.16 NEUROHORMONES INVOLVED IN PHOTOPERIOD- AND OPIATE-INDUCED PROLACTIN SECRETION. L. S. Leshin*, D. Kuehl*, K. K. Schillo*, and G. L. Jackson* (spon: E. J. Roy), Department of Veterinary Biosciences, University of Illinois, Urbana, IL 61801

In many species circulating prolactin concentrations can be elevated or depressed by exposure to long or short daily photoperiods, respectively. The purpose of this study was to determine which neurohormones mediate photoperiod-induced changes in prolactin secretion. Two groups of mature ovariectomized ewes were kept in photochambers under either short (8L:16D) or long (16L:8D) photoperiods for 60 to 90 days. Push-pull cannulae were stereotaxically positioned into the hypothalamic median eminence. After a 6- to 10-day recovery period, the ewes were subjected to hypothalamic perfusion and blood was collected from the jugular vein. Perfusate (21 µl/min) was collected continuously and divided into 15-minute fractions. Blood was collected from the jugular vein at 15-minute intervals starting 7.5 minutes after onset of perfusion. Perfusates were assayed for thyrotropin-releasing hormone (TRH) by radioimmunoassay and biogenic amines by HPLC-EC. Blood samples were assayed for prolactin. The first 1-2 hours of perfusion was a stabilization period. During the next 2 hours, saline was infused into one jugular vein. This was followed by a 2-hour infusion of morphine (1 mg/kg/hr) to acutely stimulate prolactin secretion and to determine if the hypothalamic factors measured during basal secretion also were affected. Location of cannulae tips was verified by histologic procedures. Data from 12 animals in which either the cannulae clogged during perfusion or TRH was undetectable in over 50% of samples were excluded from analysis. Data from 5 ewes kept under each photoperiod were analyzed. Long photoperiods significantly ($P < 0.01$) increased prolactin concentrations. Morphine significantly increased ($P < 0.01$) prolactin concentrations in sheep exposed to both short and long photoperiods (8L-Saline 51 ± 6 vs 8L-Morphine 175 ± 12 ng/ml; 16L-Saline 149 ± 13 vs 16L-Morphine 648 ± 71 ng/ml). TRH concentrations varied greatly among animals and may in part reflect cannulae location. Mean TRH levels tended to be higher under long photoperiods and during morphine treatment ($P < .10$) under both short and long photoperiods (8L-Saline 5.8 ± 0.7 vs 8L-Morphine 7.9 ± 0.9 pg/15 min; 16L-Saline 8.7 ± 1.3 vs 16L-Morphine 11.3 ± 2.1 pg/15 min). Detection of biogenic amines was quite variable and insufficient for pooled analysis. The metabolites DOPAC, HVA, and 5 HIAA were most commonly detected. Dopamine was rarely detected in samples that also contained TRH. The data suggest that neither photoperiod-induced nor opiate-induced increases of PRL are readily explained by enhanced TRH secretion from the median eminence. (USPHS Grant HD13037)

379.17 TIME DEPENDENT EFFECTS OF URETHANE ANAESTHESIA ON MILK EJECTION AND PLASMA PROLACTIN CONCENTRATIONS IN LACTATING RATS. D.W. McKay and K. Brown-Grant*. Fac. of Med., Memorial University of Nfld., St. John's, Newfoundland, Canada A1B 3V6.

Lactating rats anaesthetized with urethane (U) 180 minutes prior to pup application milk eject at regular intervals and show modest increases in plasma prolactin (PRL) concentrations over the next two hours. Similar rats anaesthetized with U plus xylazine (X) and suckled 30 minutes later exhibit milk ejections at rates double that observed in rats treated with U alone (McKay & Brown-Grant, Neurosci. Abstr. 9: 707, 1983). Despite the persistence of milk ejections and a continuous suckling stimulus over 120 minutes, concentrations of plasma PRL decreased rapidly.

The present study was performed to compare the effects of U alone and U plus X on the occurrence of milk ejection when pups were applied 30 minutes after the induction of anaesthesia. We also hoped to determine the effects of U and X on plasma PRL concentrations before and during suckling.

Long-Evans rats were anaesthetized with either 1.2g U/kg i.p. or 0.8g U/kg i.p. plus 10 mg X/kg i.m. on day 14 of lactation, and 10 hungry pups were applied to the nipples 30 minutes later and allowed to suckle for 120 minutes. Episodic release of oxytocin and the subsequent milk ejection (ME) were monitored by observing the synchronized stretch reactions of the suckling pups. In some animals, blood samples were taken through an intracardiac catheter which had been implanted on day 8 of lactation.

With U + X, all rats (n=10) had repeated milk ejections and the onset of ME occurred on average 2.4 minutes after pup application with an average incidence of 16 ME's for the 120 minute period of suckling. Of the animals treated with U alone, 8 of 11 had no ME's at all and the others had 1,1 and 2 respectively. There was no initial rise in PRL nor did PRL increase during suckling. Doses of 0.8g U/kg alone or 10 mg X/kg alone were not sufficient to anaesthetize lactating rats and we could not examine the ME reflex. X treatment led to elevated plasma PRL concentrations 15 minutes later (18 vs. 62 ng RP2/ml). Saline had no effect.

These findings suggest that timing may be a critical factor in studies on lactating rats under urethane anaesthesia. Milk ejections occur and PRL concentrations increase in rats suckled beginning 180 min after injection. When pups are applied at 30 minutes after induction of anaesthesia, ME's are rare and plasma PRL concentrations do not increase. The reasons for these differences or for the apparent facilitatory effect of X are at present unknown. The lack of ME does not appear to be due to a peripheral insensitivity to oxytocin. A bolus injection of 0.5 mU of oxytocin i.v. consistently induced stretch reactions in pups suckling either a U or U + X treated mother.

379.18 CIRCULATING GROWTH HORMONE (GH) LEVELS AND THE THYROTROPIN (TSH) RESPONSE TO THYROTROPIN-RELEASING HORMONE (TRH) IN DEPRESSED MEN. V.S.Wahby,M.D.,Ph.D., F.Saddik*, M.D., E.L.Giller,M.D.,Ph.D. and J.W.Mason,M.D.* VA Medical Center and Yale University, West Haven, CT 06516

PURPOSE: To find out whether circulating GH levels are related to a blunted TSH response to TRH in depressed men.

BACKGROUND: GH, via a short-loop feedback, may increase central somatostatin release, which may cause blunting of TSH response to TRH. Also, depression may be associated with blunting of that TSH response. It would be interesting to find out whether GH is implicated in this phenomenon of blunting of TSH response in depressed subjects. An initial step to evaluate this possibility is to see if there is a relationship between circulating GH levels and TSH response to TRH in this clinical condition.

METHODS: Fasting a.m. blood GH levels were determined and TRH tests (500 ug IV) were performed on 26 euthyroid men with major depressive disorder. None of the patients were diabetic, acromegalic, alcoholic, cirrhrotic or with other systemic illnesses. The incidence of depression was diagnosed by the Research Diagnostic Criteria (RDC) and its severity measured by the Hamilton Depression Scale (HDS). GH, TSH and thyroid hormones were measured by radioimmunoassay.

RESULTS: $\Delta$ TSH Maximal was 5.9 $\pm$ 0.4 uU/ml (mean $\pm$ SE) and mean basal GH level was 0.9 $\pm$ 0.2 ng/ml. No significant correlation was found between these 2 parameters, nor between GH levels and HDS ratings.

CONCLUSIONS: Basal circulating GH levels did not correlate with TSH response to TRH in depressed men. It is tempting to speculate that GH does not play a role in influencing TSH response to TRH in depression. However, it is not known whether basal serum GH levels accurately reflect its secretory rates in this clinical condition. Further work is needed to assess this finding as well as GH secretory rates in larger groups of depressed patients.

379.19 EFFECTS OF SHORT-TERM EXPOSURE TO GONADAL STEROIDS ON GROWTH HORMONE (GH) SECRETORY PATTERNS IN ADULT MALE AND FEMALE RATS. W.J. Millard, T.M. Badger*, T.O. Fox and J.B. Martin. Departments of Neurology and Gynecology, Mass. Gen. Hosp., Boston, MA 02114.

The pattern of GH secretion in the rat is sex-dependent. Males display a low-frequency, high-amplitude GH secretory pattern with bursts of GH occurring every 3-4 hrs and separated by prolonged GH trough periods where GH levels are very low or undetectable. Females show a high-frequency, low-amplitude pattern of GH secretion with pulses of GH occurring every 1-2 hrs. Individual peak amplitudes are lower and GH trough values higher and of shorter duration than those of male rats. We have previously found that long-term exposure (6-8 weeks) to gonadal steroids can alter GH secretory patterns in adult animals. In the present study we investigated whether short-term exposure to gonadal steroids can also modulate the expression of the GH secretory pattern. Adult intact or castrate male and female rats were bled via an indwelling atrial catheter for 8 hr (900-1600 hr). Immediately following the first sampling period males were given a subcutaneous 5 mm estrogen (E)-filled Silastic implant mixed 1:1 with cholesterol and females received a 15 mm testosterone (T) implant. Animals were sampled weekly for two weeks, steroid-filled capsules were removed; and then animals were sampled weekly for an additional two weeks. Although E exposure did not alter the periodicity of the GH secretory pattern in intact males, it consistently elevated GH trough levels. The effect on GH peak amplitudes was variable, with some animals showing reduced peak amplitudes. In intact females, exposure to T transformed their GH patterns into male-like GH secretory patterns. This occurred within 1 week. One week after removal of the steroid implants the homotypic GH secretory patterns again were observed in both intact male and female animals. Castration did not alter the pulse frequency of respective GH secretory patterns but did elevate GH trough levels in males and lowered trough levels in females. Within 2 weeks of E exposure the male GH secretory pattern was transformed to a female-like secretory pattern in castrate males. Like intact females, castrate females exposed to T displayed GH secretory patterns typical of males. However, unlike that of intact males and females, the GH secretory patterns in castrate animals did not revert back to their preexisting GH secretory patterns. Instead, the female GH secretory pattern persisted in castrate males given E and the male pattern persisted in castrate females given T for at least 2 weeks after removal of the steroid capsules. The contrasting data between intact and castrate animals suggest that the effects of T override those of E. T most likely acts within the hypothalamus to modulate the phasic release of somatostatin and/or GH-releasing factor. E effects both the pituitary and the hypothalamus, however, the central effects of E are apparent only in the absence of T. Supported by PHS grants AM26252, HD18656 and HD17364.

379.20 HYPOTHALAMIC SOMATOSTATIN IN GENETICALLY OBESE ZUCKER RATS. J.A. Finkelstein, L.Murray*, C.Centers* and M.Berelowitz. Dept. of Anatomy, Northeastern Ohio Univ. Col. Med., Rootstown, OH 44272 and Div. of Endocrinology/Metabolism, Univ. of Cincinnati Col. Med., Cincinnati, OH 45267.

The role of somatostatin (SOM) in the decreased growth hormone levels observed in genetically obese Zucker rats has been investigated in a series of experiments. In a previous study (DonCarlos et al, Neurosci. Abst., 1983) it was found that the number of SOM-immunoreactive hypothalamic cell bodies is similar in obese and lean rats, and that the level of SOM immunoreactivity in hypothalamic blocks from obese and non-obese rats is not different when measured by radioimmunoassay (RIA). Our first experiment was designed to investigate the possibility of subtle SOM differences in discrete hypothalamic regions. Eleven nuclear groups as well as the median eminence were micro-punched from 300u sections of hypothalamus from five obese and five lean Zucker rats. There was no difference in SOM concentration in any area studied. In a second experiment, in vitro dynamic SOM release from blocks of hypothalamic tissue was measured (six obese and six lean rats). After preincubation to achieve stable basal SOM release, the incubation medium (Krebs ringer bicarbonate buffer) was changed every six minutes for a total incubation time of 36 minutes. The protocol included one basal period followed by a period of stimulation, then four recovery periods. Basal SOM release was greater from hypothalami of obese (32.5pg/M) than from hypothalami of non-obese rats (26.7). Stimulation by 60 mM potassium led to increases in SOM release from hypothalamic blocks of both obese and non-obese rats, but the increase was greater from obese hypothalami (225.7) versus non-obese hypothalami (162). During the subsequent recovery periods SOM release decreased in both groups, with release from obese hypothalami remaining elevated above lean hypothalami until the final period. ANOVA showed significant differences between obese and non-obese rats. These in vitro data suggest that in vivo SOM release from the hypothalamus of obese rats may be chronically elevated. In such a situation, a change in the level of SOM receptors in the pituitary would be hypothesized. In a preliminary experiment, SOM pituitary receptor concentration in obese rats was about one-third of that seen in the lean animals, with no concomitant change in receptor affinity. Downregulation of SOM pituitary receptors, resulting from a chronically elevated hypothalamic SOM release, could be compatible with our failure to observe significant differences in SOM concentration in the hypothalamus of obese vs. non-obese rats, if accompanied by increased hypothalamic SOM synthesis. Chronically elevated SOM tone could also explain previous findings of decreased pituitary resposiveness to growth hormone releasing factor and SOM in obese Zucker rats (Berelowitz et al, Endocrine Soc. Abst.,1983) and obese human subjects (Williams et al, NEJM, 311,1984). Supported by NIH grants RR05806 and AM30686.

380.1 UPTAKE OF PROLINE AND PIPECOLIC ACID BY CULTURED NEURAL CELLS. J. G. Ortiz, Dept. of Pharmacology, School of Medicine, Univ. of Puerto Rico. 00936

Proline and pipecolic acid are normal constituents of the mammalian brain (Giacobini 1983). These imino acids have been shown to have properties, such as high affinity synaptosomal uptake, which are consistent with their possible role as neurotransmitters/neuromodulators (Giacobini 1983). However, it still remains to be shown whether they represent two independent neuromodulatory systems.

The uptake of $^3$H-proline (Pro) and $^3$H-pipecolic acid (PA) has been examined in three neural cell lines; N-18 (mouse neuroblastoma) PC-12 (rat pheochromocytoma) and rat glioma (C-6) in order to characterize the uptake (and transport) mechanism(s). Confluent cultures were washed with and preincubated for 1 hr. in Earle's Balanced Salts Solution, after which labelled imino acid was added. At different times thereafter, cultures were washed twice with ice-cold phosphate buffered saline (PBS). Radioactivity was determined in 0.5 M NaOH cell extracts after neutralization with glacial acetic acid.

The uptake of proline and pipecolic acid by these cell lines, is linear for at least 25 min. Experiments with a wide range of concentrations ($10^{-6}$ - $10^{-3}$ M) suggest the presence of one uptake system. The uptake of these imino acids appears to require $Na^+$ and is ouabain-sensitive. Detailed kinetic analysis shows that N-18 cells are capable of taking up both proline and pipecolic acid with similar affinities (1.4 mM and 6.0 mM respectively). On the other hand, PC-12 and C-6 cells have higher affinity for proline than for pipecolic acid. The different characteristics of the proline and pipecolic acid uptake system by these cell lines may be useful in the understanding of the possible role(s) of these imino acids in the Central Nervous System.

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380.2 CYTOCHEMICAL VISUALIZATION OF FREE RADICAL FORMATION IN SYMPATHETIC NEURONS EXPOSED TO A BRIEF PERIOD OF STARVATION. J.C. Saez*, J.A. Kessler+, M.V.L. Bennett and D.C. Spray. Depts. of Neuroscience and Neurology+, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

Substrate deprivation occurs during pathological conditions such as ischemia, perhaps leading to tissue injury as a consequence of free radical (FR) generation. Involvement of FR generation in ischemia of the nervous system has been difficult to prove; FRs are extremely reactive and have such short lifetimes (<1 s) that they are difficult to detect, and substrate deprivation (starvation) in ischemia is complicated by hypoxia. Starvation is easily produced in superior cervical ganglion cells maintained in culture (Saez et al., Soc. Neuroscience Abstr. 10: 887, 1984). A 10 min exposure to glucose free saline kills 45% of the cells. We now report that certain chromophores that are reduced or oxidized by FRs (depending on their redox potentials) permit the visualization of FR generation in single neurons during and after starvation. In one procedure cells were incubated for 10 min in glucose free saline with nitro blue tetrazolium (NBT). During starvation many cells turned blue due to reduction of NBT which is pale yellow in its oxidized state. Intracellular superoxide anions were involved, since the color change was blocked by intracellular superoxide dismutase, internalized by presoaking along with 50 mM $K^+$. A second approach was to load cells with the nonfluorescent indicator 2', 7'-dichlorofluorescin ($Cl_2FH$) by soaking in its diacetate ester. The ester enters the cells and $Cl_2FH$ is then released by action of intracellular esterases and retained because of its polar nature. In starved cells $Cl_2FH$ is oxidized to the highly fluorescent 2', 7'-dichlorofluorescein ($Cl_2F$), indicating that oxidants are generated intracellularly. The number of cells showing reduction of NBT correlated with the mortality rate determined by trypan blue incorporation in parallel experiments. Cells showing $Cl_2F$ fluorescence subsequently lost their fluorescence and incorporated trypan blue, indicating loss of the surface permeability barrier and death. Since generation of FRs and other oxidants is oxygen dependent, it is unlikely that they are generated during hypoxia (another consequence of ischemia). Indeed both reactions were substantially less frequent when cells were maintained in a $N_2$ environment for 10 min, consistent with the lower mortality rate following $N_2$ treatment (7%). Based upon our separation of starvation and hypoxic aspects of ischemia and cytochemical techniques that allow indirect visualization of FRs during these insults, we propose that FR generation is at least partially responsible for the deleterious changes induced by substrate deprivation.

380.3 MACROPHAGES ENHANCE THE PROBABILITY OF NEURONAL SURVIVAL AFTER AMPUTATION OF DENDRITES. C.R. Gardner* and G.W. Gross (SPON: D.J. Eder). Department of Biology, Texas Woman's University, Denton, TX 76204.

We have previously reported that elicited peritoneal macrophages (MPs) cocultured with neurons were attracted to lesions along neurites. The lesions were performed with laser microbeam cell surgery at a wavelength of 337nm and constituted both complete neurite transections as well as localized neurite damage resulting only in cytoplasmic pinching at the 2.2μm diameter irradiation site (Gardner and Gross, Soc. Neurosci. Abstr. 9:594, 1983). Complete neurite transections usually triggered phagocytic activity at the lesion suggesting that MPs might interfere with neuronal recovery. However, contrary to expectations, the presence of MPs significantly enhanced the survival of neurons after surgery. At a lesion distance of 50μm from the soma, the percent cell survival increased from 31±3% (SEM) in normal MEM with 10% horse serum (MEM 10), to 64±5% in MEM 10 supplemented an average of 7 days earlier with $2x10^6$ MPs per 4ml medium (28cm$^2$ culture area). At a lesion distance 100μm from the soma, survival increased from 53±6% to 88±5%. These highly significant increases in percent cell survival are based on a total of 120 neurite amputations at each lesion distance.

At the present time, it is unclear whether macrophage contact with the lesion is essential for enhancing neuronal survival. Some preliminary experiments have indicated that we may be dealing with a soluble but labile factor secreted by MPs.

380.4 ETHANOL-INDUCED LIPID PEROXIDATION IN BRAIN MEMBRANES. F. Ahmad,* D-Z. Lee,* D. Cowan* and A.Y. Sun. Sinclair Comparative Medicine Research Farm, Biochem. Dept. and Dept. of Phys., Univ. of Missouri, Columbia, MO 65203.

Brain membranes contain a high proportion of polyunsaturated fatty acids and are more susceptible to free radical attack. We have shown that ethanol enhanced lipid peroxidation in brain microsomal membranes in vitro (Ahmad et al., Trans. Am. Neurochem. Soc. 15, 1985). By using N-t-butyl-2-phenyl-nitrone (PBN) to trap the free radicals formed and examining the spin signal with an electron spin resonance (ESR) spectrometer, we found that brain microsomes are active in generating free radicals in the presence of $Fe^{++}$. Ethanol (0.5 M) added to the incubation medium containing microsomes and $Fe^{++}$ greatly enhanced the free radical formation. When animals were treated with an acute dose of ethanol (6 g/kg) and sacrificed at 3 or 24 hr after ethanol administration, no spin signals were detected in either liver or brain of control and alcohol-treated animals. However, by employing a free radical-generating agent, $CCl_4$, we were able to detect free radical formation in both brain and liver using PBN as trapping agent. An acute dose of ethanol enhanced the $CCl_4$-induced free radical formation in liver and brain at 3 or 24 hr after alcohol administration. The results indicate that ethanol per se may not stimulate free radical formation. However, ethanol may enhance free radical formation by perturbing the membrane acyl groups, thus exposing viable membrane sites for free radical attack. An increase in the level of intracellular lipofuscin in brain has been reported in the chronic alcoholic patients. It is possible that alcohol drinking may enhance age pigment formation and accelerate aging through elevated lipid peroxidation and other pro-oxidation reactions due to free radical attack. (Supported in part by grant No. AA02054 from DHHS.)

380.5 INTERRELATION OF BICARBONATE & AMMONIA CHANGES IN SPREADING DEPRESSION. R.P. Kraig & A.J.L. Cooper*. Department of Neurology, Cornell University Medical College, New York, New York 10021.

Spreading depression (SD) is a reproducible and transient perturbation of brain characterized by biochemical and biophysical changes similar to those of ischemia. Hence, SD may be a useful pathophysiologic event through which to study the mechanisms by which brain regulates $[H^+]$ in compromised states such as ischemia. SD is associated with an alkaline, then acid going shift in $[H^+]_o$ (Kraig et al., J Neurophysiol 49:831, 1983). In neocortex, lactate accumulates to 7 mmol/kg during SD (Mutch & Hansen, JCBF & Met 4:17 1984). How volatile $H^+$ buffers (i.e. $HCO_3^-$ & $NH_3$) respond to this acid load is unknown.

Rats were anesthetized with halothane and spontaneously ventilated; warmed to 37°C; and an artery cannulated. Parietal cortex was exposed and superfused with Ringer. Arterial pressure, pH, $PCO_2$, $PO_2$, and glucose were stabilized. SD was elicited by a 1-3 sec, 100 Hz stimulus to nearby cortex. $[H^+]_o$ and either $PtCO_2$ or $[NH_3]$ were monitored in pairs. $H^+$ microelectrodes (tridodecylamine) were placed 300 µm below and $PtCO_2$ or $NH_3$ electrodes at the pial surface. To compare volatile $H^+$ buffer changes to $[H^+]_o$, we improved the response time (95%) of the $PtCO_2$ and $NH_3$ electrodes from 40 & 50 sec to 5 & 10 sec, respectively. In addition electrodes responded linearly between 1-760 mm Hg and 1 µM-1 mM, respectively.

Temporal changes were compared to the negative dc shift of SD. $[H^+]_o$ changes began simultaneously with the dc shift and consisted of a brief alkaline, then acid shift. $[H^+]_o$ was 7.30±0.01 pH (n=33) before SD and became more acid in 9±1 sec to reach a peak of 6.93± 0.02 pH (36±1 sec) before returning to baseline 11.9±0.8 min later. $PtCO_2$ changes began 11±2 sec (n=12) after the dc shift started; reached a peak in 37±2 sec; and returned to baseline in 6.6±1.3 min. $[HCO_3^-]_o$ changes were calculated from these measured variables and showed that $[HCO_3^-]_o$ first rose with the alkaline spike and then reached a low of 13.6±0.6 mM (between 9-37 sec after the dc shift) and returned to baseline in 11.9±0.8 min. $[NH_3]$ changes were more delayed and prolonged. SD initiation produced a small rise in $[NH_3]$ from 2.3±0.1 µM (n=20) but with SD $[NH_3]$ rose rapidly to reach a peak of 4.4±0.3 µM 1.8±0.1 min after the dc shift. Furthermore, 20.8±2.1 min elapsed before $[NH_3]$ returned to baseline.

These results show that during SD rapid and focal changes in $[HCO_3^-]_o$ and $[NH_3]$ can occur in brain. The delayed changes in $[NH_3]$ imply that they are a response to the rise in $[H^+]$ and $PtCO_2$. If so, the $[NH_3]$ changes during SD could influence brain acid-base homeostasis directly through physicochemical $H^+$ buffering of $NH_3$ and indirectly by $HCO_3^-$ production from glutamine metabolism. (Supported by NS-19108, NS-003346, and a Teacher Investigator Award to R.P.K.)

380.6 APOLIPOPROTEINS SERVE AS MARKERS IN THE CSF FOR DEMYELINATING NEUROPATHY. S. S. Bock*, G. J. Snipes, J. J. Norden, & J. A. Freeman (SPON: A. Burt) Dept. of Anatomy, Vanderbilt University, Nashville, TN 37232.

In demyelinating conditions produced by stroke, trauma, and various progressive neurological diseases, myelin lipids must be removed from the site of demyelination and redistributed. We have recently discovered that part of this lipid shuttling mechanism involves the induction and secretion of apolipoproteins by non-neuronal cells within the central nervous system (Snipes et al., PNAS, 1985). We have obtained direct evidence, by immunohistochemistry, that apolipoprotein E (Apo E) accumulates around degenerating fiber tracts in the rat brain. As a result of CNS injury apolipoproteins and lipids form large macromolecular lipoprotein complexes which we have isolated by density gradient ultracentrifugation. While characterizing these brain derived lipoprotein complexes, we have identified an additional apolipoprotein, Apo Al, produced by non-neuronal cells in response to injury (Snipes et al., this volume). Similar lipoprotein complexes exist in serum. An interesting property of these serum lipoproteins is that systemic lipid dynamics are reflected in the quantity and composition of individual apolipoproteins. Therefore, since we now know that the brain responds to injury by the secretion of apolipoproteins, we tested whether demyelinating CNS neuropathies might be reflected in the amount and composition of apolipoproteins found in cerebrospinal fluid (CSF). To do this, we used anti-Apo E and anti-Apo Al antibodies to quantitate, by western blot analysis, the levels of these apolipoproteins in the CSF of brains of normal rats and of rats that have received different injuries. In preliminary experiments, we found that CSF Apo E levels were unchanged, but that Apo Al levels underwent a significant increase during demyelination following traumatic injury to the CNS. These findings raise the possibility that specific neurological diseases may be diagnosed and the extent of active degenerative neuropathy associated with such conditions as demyelinating disease, stroke, and trauma can be detected and quantitated by monitoring the level of apolipoproteins in the CSF. This would provide a valuable clinical tool. (Supported by NIH Grant NS18103 and NEI Grant EY01117 to J.A.F.)

380.7 RELATIONSHIP BETWEEN AXONAL AND MYELIN SHEATH PARAMETERS FOLLOWING AXONAL ATROPHY PRODUCED BY ACRYLAMIDE INTOXICATION. B. G. Gold*†, J. W. Griffin§ and D. L. Price§. †The Johns Hopkins University School of Hygiene and Public Health, §The Johns Hopkins University School of Medicine, Baltimore, MD 21205

Myelin sheath thickness is, in general, closely related to axonal caliber and internodal distance. However, it is not clear which axonal parameters are the most significant determinants of sheath thickness. In the present study, we have used a model of proximal axonal atrophy produced by repeated acrylamide (AC) administration to separate the effects of axonal area (AA) and axonal perimeter (AP) on the thickness of the myelin sheath.

Three-week old Sprague-Dawely male rats received daily intraperitoneal injections of AC (30 mg/kg) for 24 days. At seven weeks of age, animals were perfused, along with age-matched controls, with 5% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.3). Morphometric analysis of the L5 dorsal root ganglia was performed using light microscopic prints (200-300 fibers/section) to determine axonal diameters and areas. Histograms of axonal diameter and area were constructed and mean values determined for the largest 25% of fibers. Electron microscopic prints of randomly photographed fibers (4-5 fibers/grid) were used to determine the AP, as well as the numbers of neurofilaments (NF) and myelin lamellae. From this data, we calculated AA, NF density, and the length of the "unrolled" sheath, or myelin spiral length (MSL) for each fiber. Osmium-postfixed sciatic nerves were teased in glycerol for measurement of internodal distances.

Histograms of axonal diameters were significantly ($p < 0.05$; Mann-Whitney U-test) shifted to the left in AC-intoxicated animals with little or no apparent loss of fibers at this level. Mean axonal diameters and areas for the largest 25% of fibers were 23% and 41%, respectively, of control values. In intoxicated animals, NF density was increased (120 vs. 175 NF/$\mu m^2$) to levels comparable to those observed in AC-induced proximal swellings, and myelin sheaths appeared relatively thick for axonal caliber. In normal rats, MSL was linearly ($r=0.94$) related to AP; a less significant ($r=0.87$) correlation was obtained between MSL and AA. AC-intoxicated rats also demonstrated a significant ($r=0.91$) relationship between MSL and AP but no relationship between MSL and AA ($r=0.14$). In AC-intoxicated rats, the slope of the relationship between MSL and AP was significantly ($p < 0.05$) less (135 vs. 99; control and AC-intoxicated, respectively), and teased fibers had shorter internodal lengths.

The results demonstrate that MSL remains linearly correlated with AP in an experimental model where AA is severely reduced. It is suggested that the reduced relationship, i.e., slope, between MSL and AP is a result of a failure of longitudinal and radial growth of fibers in these young AC-intoxicated animals.

380.8 HUMAN GLIOMA- AND CNS METASTASIS-DERIVED TUMOR CELL GROWTH REGULATION AND INTERACTIONS WITH NORMAL CELL POPULATIONS. B.H. Smith, D. Parker*, J. Galicich*. Dreyfus Medical Foundation and Memorial Sloan-Kettering Cancer Center, New York, NY 10022.

The established human glioma-derived cell line U251, new cell lines derived from metastatic brain tumors, as well as human dural-derived fibroblast lines and a bovine endothelial cell line have been utilized to compare neoplastic glial cell growth regulation, as well as the interactions between these tumor cells and normal vascular cells and fibroblasts. Initial cultures were established from surgical explants in MEM with NEAA supplemented with 10% fetal calf serum (GIBCO) in a 3.5-5.0% CO2 atmosphere at 37C. Routine mycoplasma screening is carried out. Passage numbers for U251 were P16-35 and for the other lines P1-6. Feeder layers were dural fibroblasts irradiated with 3000 rads. Standard growth curves were established for each cell line.

Growth regulatory effects of feeder layers, collagen, epidermal growth factor (EGF), dibutyryl cAMP (10-3,-4M), dimethylformamide (DMF) (up to 1.5%), phenytoin (PHT) (1-100mcg/ml), as well as extracellular K+, Na+, and Ca2+ have been evaluated in all lines. DBcAMP produced growth retardation (up to 70% at 10-3M) in all lines tested, although the effect was more marked in the tumor lines (U251, other gliomas, and lung carcinoma). PHT (50-100mcg/ml) had a tumor line effects (70 and 50%) at 7-10 days. DMF had a striking inhibitory effect (90%) by day 7 in U251 and induced morphological changes as well. EGF has been without effect on the glioma-derived lines, but does enhace fibroblast growth. Fibroblast feeder layers appear to inhibit U251 to a modest degree, but may accelerate the lung cell growth. The inhibitory effects of both DBcAMP and PHT are consistent with alterations in ionic flux, in particular Na+ and Ca2+ influx for PHT.

The availability of both normal and neoplastic cell lines provides a useful in vitro model for the study of primary and secondary tumor cell growth regulation normal -tumor cell interactions and differing invasive potentials in the CNS.

380.9 NEURAL CELL ADHESION MOLECULE (N-CAM) ACCUMULATES IN DISEASED (DENERVATED) HUMAN MUSCLE FIBERS. N.R. Cashman[1], J. Covault[2], R.L. Wollman[1], and J.R. Sanes[2], [1]Dept. of Neurology, Univ. of Chicago, Chicago, IL 60637, and [2]Dept. of Anatomy and Neurobiology, Washington Univ. School of Medicine, St. Louis, MO 63110.

N-CAM, a glycoprotein present on neuronal surfaces, is thought to mediate a variety of intercellular adhesive interactions in the nervous system (Rutishauser, Nature 310, 549, 1984). While most intensively studied as a neuronal protein, N-CAM is also transiently expressed in muscle: it is present on embryonic myotubes, but is lost during development and is virtually absent from adult muscle fibers. However, denervation of adult rat, mouse, or chicken muscle leads to the reappearance of N-CAM on the surface and within the cytoplasm of denervated fibers (Covault and Sanes, PNAS, 1985). We report here that N-CAM also appears in muscle fibers of patients with denervating diseases.

Biopsies of deltoid and gastrocnemius muscles, obtained for clinical diagnostic purposes, were frozen and cross-sectioned in a cryostat. Sections were stained with affinity-purified antibody to chicken N-CAM (known to cross-react with mammalian N-CAM; Covault and Sanes, op cit.) and fluorescein-second antibody, then examined with epifluorescent illumination. N-CAM immunoreactive fibers were observed in muscles from patients with several diseases known to involve denervation: amyotrophic lateral sclerosis (ALS), infantile spinal muscular atrophy (Werdnig-Hoffman disease), sensory-motor neuropathy, and progressive denervating post-polio syndrome. In each case, most of the stained fibers were likely to have been denervated, as judged by their small diameter. However, not all small fibers were stained, nor were all stained fibers small. In particular, a histologically normal biopsy from an ALS patient displayed scattered N-CAM positive fibers, suggesting that N-CAM-staining may detect recently denervated fibers that have not yet atrophied. N-CAM-positive fibers were rarely detected in normal muscle or in muscles from patients with disorders that are thought not to involve denervation (rheumatologic type II fiber atrophy, and myotonia). No fibers were stained when normal rabbit Ig was substituted for anti-N-CAM. Thus, N-CAM accumulates selectively (although perhaps not exclusively) in denervated human muscle fibers. N-CAM immunohistochemistry may therefore be a useful technique in the differential diagnosis of diseases of the motor units. Furthermore, in that some synaptogenetically important nerve-muscle interactions may be mediated by N-CAM (Rutishauser et al., J. Cell Biol. 97, 145, 1983; Covault and Sanes, op. cit.), it will be interesting to seek diseases in which disorders of innervation are not accompanied by accumulation of N-CAM. (Supported by NIH and MDA).

380.10 PRELIMINARY EVIDENCE OF MAGNESIUM DEPLETION AND EXCESSIVE CALCIUM ACCUMULATION IN HUMAN MYOBLASTS OF DYSTROPHIC ORIGIN. Syamal K. Bhattacharya and Alice J. Crawford. Surgical Research Laboratory, University of Tennessee Medical Center, Memphis, Tennessee 38163.

Duchenne muscular dystrophy (DMD) is a progressively crippling X-linked disease characterized by biochemical, histological, and cardiac abnormalities which result in severe muscle degeneration. The membrane hypothesis suggests a generalized functional defect in the cellular plasma membranes of DMD patients which stimulates an increased cellular ingress of $Ca^{2+}$ and $Na^{+}$ and a leakage of intracellular constituents such as $K^{+}$, CK, and LDH to the extracellular environment. In previous studies, we have demonstrated that muscle Ca content is increased by 300% and muscle Mg content is depleted by 45% in patients with DMD (Neurology,32:1088,1982). Serum Ca and Mg concentrations, however, are normal in human and animal forms of muscular dystrophy (MD) while the serum CK level is extremely elevated. These biochemical abnormalities also occur to a lesser extent in patients with other neuromuscular disorders such as congenital myopathies, polymyositis, and fascioscapulohumeral and limb girdle MD. We have demonstrated that older non-ambulatory DMD patients reveal a significantly lower ($p<0.001$) muscle Mg content compared to younger ambulatory patients whose muscles are also Mg deficient. We have recently reported excessive intracellular calcium accumulation (EICA), in the absence of cellular necrosis, in the biceps and quadriceps of human fetuses at risk of DMD, with no apparent muscle Mg depletion (Neurology,34:1436,1984). This evidence indicates that muscle Mg deficiency is present in the early stages of DMD and becomes progressively severe as the disease advances, whereas EICA occurs in utero and does not change with time. Since we hypothesize that muscle Mg may have an antagonistic effect on EICA in MD, we studied the Ca and Mg contents of myoblasts, grown in vitro, collected from apparently normal human fetuses and those at risk of DMD. Myoblasts were grown in 80% Dubelco media, fortified with 10% horse serum and 10% fetal calf serum, harvested at equivalent stages of maturation, and washed twice with Ca and Mg free normal saline. Minerals were measured in the myoblasts and growth media before and after cell growth, and in the saline-wash before and after myoblast washings, to ensure an accurate comparison between normal and dystrophic myoblasts and to test for contamination or mineral uptake by the growth media or saline wash. We found a decreased Mg content and an elevated Ca content in the myoblasts of dystrophic origin as compared to the controls. To overcome possible cell counting errors, we calculated the Mg:Ca ratio and found it to be severely depressed in dystrophic myoblasts. This observation correlated well with our previous Mg:Ca ratio results in DMD patients and fetuses at risk of DMD. We conclude that in DMD: 1) EICA represents one of the earliest detectable biochemical changes 2) Muscle Mg depletion is progressive, whereas EICA does not change with disease advancement 3) Mg depletion and EICA also occur in human myoblasts of dystrophic origin.

380.11 CELL TRANSPLANT TREATMENT FOR MUSCLE DISEASES. P.K. Law and T.G. Goodwin*, Depts. of Neurol., Physiol./Biophys., Univ. of Tennessee, Memphis, TN 38163

Muscle development and complementation mechanisms were studied after genetically normal myoblasts were injected into growing and regenerating dystrophic muscles. Applicability of this new technique to alleviate muscle weakness in hereditary myopathies was tested in dystrophic mice. We showed that the procedure significantly improved the structure and function of dystrophic muscles.

Dystrophic cells degenerated because of the lack of the normal genome. The normal genome might become incorporated when normal donor myoblasts fused with satellite cells of the dystrophic hosts to form mosaic myofibers. In myopathies of recessive inheritance, gene products from the normal nuclei might restore the phenotype of these heterokaryotes to normal. Furthermore, surviving donor cells would develop and replace the myopathic tissue. These two mechanisms of genetic complementation were demonstrated in this study.

About $10^6$ cultured myoblasts from genetically normal mouse embryos were injected into the right soleus of 20-day-old normal or dystrophic mice. Hosts and donors were of C57BL/6J background, and were immunocompatible. Test and control muscles were compared at 6 months after injection. Survival and development of donor myoblasts in host muscles were analyzed with electrophoresis of muscle isozymes of glucose phosphate isomerase (GPI). Donor cells produced GPI-1C. Host cells produced GPI-1B. Presence of both isozymes and/or GPI-1BC substantiated genetic mosaicism in the test dystrophy solei.

Table 1 shows that the test dystrophic soleus generated significant greater twitch (Pt) and tetanus (Po) tensions than the contralateral, unoperated muscle. The test dystrophic soleus also showed a reduction in half-relaxation time (1/2 RT) towards normal. It had more fibers with high resting potentials (74.6 ± 6.2 SD (48)) than its control (68.2 ± 6.0 (48)). It showed significant improvement over its control histologically.

TABLE 1

| | Normal | | Dystrophic | |
|---|---|---|---|---|
| | Test | Control | Test | Control |
| Pt (gm) | 3.2 ± 0.3 | 3.0 ± 0.5 | 2.8 ± 0.5 | 2.1 ± 0.6* |
| Po (gm) | 18.5 ± 3.1 | 14.2 ± 3.2* | 13.1 ± 2.6 | 9.0 ± 2.7* |
| 1/2RT(msec) | 16.8 ± 3.8 | 15.9 ± 3.0 | 19.8 ± 3.5 | 24.9 ± 3.5* |

Mean ± SD of 5 normal and 4 dystrophic mice studies at 37°C in vivo with nerve stimulation. Paired t-test was used. *, significant at P 0.01 level.

We have hereto demonstrated the beneficial effects and the genetic complementation mechanisms of injecting normal myoblasts into dystrophic muscles. (Supported by USPHS NS-20251 and MDA)

381.1 WHEAT GERM AGGLUTININ-RICIN A-CHAIN CONJUGATE IS A POTENT CYTOTOXIN IN VITRO BUT IS LESS ACTIVE AS A SUICIDE TRANSPORT AGENT IN THE CNS T.N. Oeltmann & R.G. Wiley. Medicine and Neurology Depts., Vanderbilt Univ. Med Sch and VAMC, Nashville, TN, 37203.

The disulfide conjugate of wheat germ agglutinin to ricin A-chain (WGA-SS-RTA) is active as a suicide transport agent when injected into the vagus nerve of a rat. It is retrogradely transported to vagal sensory and motor neuron cell bodies and destroys these neurons (Abstracts Soc.Neurosci., 10:352,1984). In the present study, we sought to determine if WGA-SS-RTA was an effective suicide transport agent in the CNS. WGA-SS-RTA was synthesized and purified as previously described (above). The hybrid toxin was dissolved in PBS containing $NaN_3$ and N-acetylglucosamine and pressure microinjected (0.5-1 ul containing 2-10 ug toxin) into the caudate nucleus of anesthetized adult male rats using stereotaxic technique. After 2 days-2 weeks survival, rats were reanesthetized and transcardially perfused with phosphate buffered aldehyde fixative. Brain sections were cut out of 30% sucrose in a cryostat and processed for indirect peroxidase immunohistochemistry using the Vectastain (Vector Labs, Burlingame, CA). Rabbit primary antiserum to ricin A-chain was raised in our lab; anti-WGA was purchased (Vector). This same immunohistochemical procedure demonstrates retrograde transport WGA-SS-RTA by vagal neurons. Alternate sections were stained with cresyl violet (Nissl stain), and 4 rats were processed for combined cresyl violet and catecholamine histofluorescence using the formaldehyde-aqueous glutaraldehyde (FAGLU) technique. Cresyl violet and histofluorescence sections demonstrated definite but incomplete cell loss in the substantia nigra pars compacta (SN) ipsilateral to the caudate injection in 6 of 10 rats; 4 showed equivocal cell loss. Immunohistochemical staining was positive for WGA in 4 of 4 rats but negative for ricin A-chain in 4 of 4. In order to understand why WGA-SS-RTA was not more effective as a suicide transport agent, the toxin was tested for its ability to inhibit protein synthesis in vitro. Neuroblastoma cells (2A) in monolayer culture or hematopoietic stem cell line (K-562) in suspension culture were incubated at 37°C for 18 hrs with WGA-SS-RTA and then pulse labelled with a radioactive amino acid mixture for 2 hrs. Radioactivity incorporated into TCA precipitable material was quantitated by liquid scintillation counting. WGA-SS-RTA was highly effective in blocking protein synthesis in both cell lines ($LD_{50}$=0.2 nM for K-562). These results indicate that WGA can function effectively as a carrier under certain conditions (in vitro) but WGA-SS-RTA is not efficient in delivering ricin A-chain to neuron cell bodies in vivo presumably due to premature dissociation of the hybrid toxin. (Supported by VA Merit Review Award.)

381.2 GLYCOSIDASE ENZYME DIGESTION AND LECTIN CYTOCHEMICAL STUDIES OF AXONALLY TRANSPORTED GLYCOCONJUGATES. C.E. Hart* and J.G. Wood* (SPON: M. Tigges). Dept. of Anatomy, Emory Univ. Sch. of Med., Atlanta, GA 30322

The carbohydrate composition of axonally transported glycoproteins in sciatic nerve of rat was studied by post-embedding lectin cytochemistry in combination with glycosidase enzyme digestions. A cold block procedure for the interruption of axonal transport was employed to selectively increase the population of anterograde moving components on the proximal side of the transport block. Electron microscopic observations revealed that a cold block applied for 6 hours to the sciatic nerve of an anesthetized rat produced an increase in axonal smooth membrane vesicles at a site directly proximal to the cold block. Axon profiles greater than 0.5 mm proximal to the cold block or axons from control nerves showed a normal distribution and density of smooth membrane vesicles. Post-embedding lectin cytochemistry of the sciatic nerve demonstrated a substantial increase in Concanavalin A (Con A), wheat germ agglutinin (WGA), and succinylated WGA binding sites in axons directly proximal to the cold block. Axons outside the cold block area or in the control nerve had very few lectin binding sites observable by light microscopy. Endoglycosidase H (endo H) digestion prior to lectin cytochemistry characterized a large population of the axonally transported Con A binding sites as polymannose and/or hybrid N-linked oligosaccharides (endo H susceptible). Also, a distinct population of neuraminidase resistant WGA binding sites was found in axons directly proximal to the transport block. The increase in smooth membrane vesicles observed at the ultrastructural level and the increase of lectin binding sites in axons proximal to the transport block support the hypothesis that a system(s) of smooth membrane inside the axon is involved in the transport of axolemmal and synaptic terminal glycoproteins from the cell soma to their cell surface destinations. Results of glycosidase enzyme digestions and lectin cytochemistry experiments suggest that many of the axonally transported glycoprotein carbohydrates are polymannose and/or hybrid N-linked oligosaccharides. This observation is especially interesting in relation to our previous reports which indicate that most lectin binding sites on the neuronal cell surface are composed of complex oligosaccharides. Supported by USPHS grant NS-17731.

381.3 SYNAPTOGENESIS AND EYE-OPENING PRECEDE ANTEROGRADE TRANSSYNAPTIC TRANSPORT OF WHEAT GERM AGGLUTININ-HORSERADISH PEROXIDASE (WGA-HRP) IN THE DEVELOPING RAT RETINO-TECTO-PARABIGEMINAL PATHWAY. S.K. Itaya Dept. of Anatomy, Univ. of Illinois at Chicago, Chicago, IL 60680.

In previous studies we have found that the lectin conjugate, WGA-HRP, undergoes anterograde transsynaptic transport in several sensory pathways in the central nervous system of both rats and monkeys. Two characteristics suggest that a highly specific neuronal mechanism is involved: 1) anterograde transsynaptic transport of WGA-HRP occurs in some, but not all, pathways, and 2) anterograde transsynaptic transport is neuron-specific, directionally-specific, and limited to synaptically connected neurons. Based on these findings, we hypothesized that the transport of WGA-HRP actually demonstrates a pre-existing, interneuronal route normally taken by an endogenous, WGA-HRP-like molecule. Such a molecule could function as a recognition signal prior to synaptogenesis, and/or as a trophic factor in the mature nervous system. In order to test this hypothesis, we have studied transport of WGA-HRP in a developing pathway, using the rat retino-tecto-parabigeminal pathway as a model. In adult experiments, intravitreal injections of WGA-HRP consistently label retino-tecto-parabigeminal terminals, thus demonstrating anterograde transsynaptic transport. Much of the rat visual system develops postnatally, so anterograde transsynaptic transport can be correlated with developmental events, e.g., eye-opening at 16 days and retino-tectal synaptogenesis, which begins at birth (Lund, R.D. & Lund, J.S., Brain Res. 41:1, 1972). Neonatal rats at 7, 16, and 22 days of age were studied. Rat pups were anesthetized and 1% WGA-HRP (Sigma) was injected into the vitreous. After 1-2 days, the animals were reanesthetized, perfused, and brain sections were processed for the TMB procedure (Mesulam, M.-M., J. Histochem. Cytochem. 26:106. 1978). In all cases there was heavy labeling of the primary visual pathways, especially in the contralateral superior colliculus. Terminal labeling in the parabigeminal nucleus, however, was not present in the 7 or 16 day old cases, but appeared only in the 22 day old. Thus, we conclude that while anterograde transport of WGA-HRP occurred in all age groups, anterograde transsynaptic transport of WGA-HRP took place only in the 22 day old rat. Our results indicate that anterograde transsynaptic transport does not occur during synaptogenesis, nor does it occur prior to eye-opening, even though the axons and synapses of the retino-tecto-parabigeminal pathway are in place (Linden, R. & Perry, V.H., J. Comp. Neur. 218:270, 1983). The results suggest that anterograde transsynaptic transport occurs only at a physiologically functioning synapse, and therefore probably has a role related to synaptic transmission. Thus, the hypothetical, endogenous WGA-HRP-like trophic factor may act as a signal indicating that a synaptic connection is intact. Supported by NIH NS21021, BRSG 5369 & 8309.

381.4 AXONAL TRANSPORT OF MONOCLONAL ANTIBODIES. T.C. Ritchie, R.H. Fabian*, J.V.A. Choate* and J.D. Coulter. Marine Biomedical Institute and Dept. of Neurology, Univ. Texas Medical Branch, Galveston, Texas.

Three monoclonal antibodies against rat brain synaptosomes, produced by conventional hybridoma techniques, were screened for their ability to undergo uptake and axonal transport in vivo. Injections of ascitic fluid or of purified IgG were made into the vitreal chamber of the eye in anesthetized rats to test for anterograde transport in retinal afferents to the contralateral superior colliculus. Retrograde transport by facial nucleus motoneurons was evaluated after injections of antibody into the mystatial vibrissal skin and musculature. Transported immunoglobulins were localized in tissue sections using a modification of the peroxidase anti-peroxidase technique. One monoclonal antibody, S-2C10, was found to undergo fast anterograde transport in retinal ganglion cells and retrograde axonal transport in facial motoneurons. The anterograde transport rate was determined to be at least 100 mm/day. Transported immunoglobulins were detectable even after injection of dilute antibody solution (0.01%), and the uptake-transport process for this antibody appears saturable. The antigen recognized by S-2C10 was shown to accumulate proximal and, to a lesser degree, distal to ligations of the sciatic nerve, suggesting that the antigen is synthesized and transported by motoneurons and/or sensory ganglion cells. Preliminary studies indicate the antigen is likely to be an intrinsic membrane protein or proteoglycan. Two other antibodies tested, S-4E9 and S-1G10, exhibited the ability to undergo retrograde transport, but only after injections at relatively high antibody concentrations ($\geq$ 1.0%). Neither of these antibodies was shown to undergo anterograde transport. The retrograde labeling produced by injections of S-4E9 and S-1G10 consisted of punctate reaction granules within the motoneuron somata. In contrast, S-2C10 was localized in neuronal perikarya, proximal dendrites and in the adjacent neuropil of the facial nucleus after retrograde transport. The uptake of both S-4E9 and S-1G10 antibodies appears to involve non-specific, fluid phase endocytosis. Uptake-transport of the S-2C10 antibody, however, appears to be mediated by specific, adsorptive endocytosis after binding of the antibody to a plasma membrane component (or components) present on both soma-dendritic and nerve terminal membranes. These results indicate that monoclonal antibodies will be useful as in vivo and in vitro probes for characterizing neuronal plasma membranes, and the composition and intracellular processing of internalized macromolecules.

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381.5 UPTAKE AND TRANSPORT OF ANTIBODIES TO WHEAT GERM AGGLUTININ BINDING GLYCOCONJUGATES. J.V.A. Choate*, T.C. Ritchie, R.H. Fabian* and J.D. Coulter. Marine Biomedical Institute, Depts. of Physiol. & Biophys., Psychiat. & Behav. Sci. and of Neurology, Univ. of Texas Medical Branch, Galveston, TX 77550.

Previous experiments have shown that the lectin wheat germ agglutinin (WGA) and antibodies to wheat germ agglutinin binding glycoconjugates (antiWGA-GC) are transported retrogradely by rat facial motoneurons. In addition, the transport of antiWGA-GC can be inhibited by incubation of antiWGA-GC with its immunogen. Since it is well established that the uptake of WGA is via adsorptive endocytosis mediated by binding to membrane "receptors," competition between WGA and antiWGA-GC would suggest that a similar process underlies uptake of the antibodies.

Polyspecific antibodies were generated in rabbits against wheat germ agglutinin binding glycoconjugates, isolated by lectin affinity chromatography from Triton X-100 solubilized rat brain synaptosomes. The antiserum (50% sol) or preimmune serum containing different concentrations of WGA was injected into the vibrissal musculature of anesthetized rats. Controls consisted of injections of antiserum, preimmune serum or WGA alone into the contralateral side. After 24 hours, the rats were anesthetized and perfused with 3.5% paraformaldehyde. Retrogradely transported antibody or WGA was localized in the facial nucleus. Retrograde transport of the antibody was blocked by WGA concentrations of 100 μM or greater. Transport of the antibody was not blocked by other lectins, including peanut agglutinin and Lens culinaris agglutinin. Retrograde transport of WGA was not blocked by the antiserum. That WGA transport is not detectably effected by the antiserum seems likely to be due to the antiserum being directed to a subset of the WGA "receptors." Comparison of the staining patterns of the antiserum and the lectin on Western blots of electrophoresed WGA binding proteins supports this conclusion. To control for the possibility that the WGA lectin blocks the antiserum transport by cross-linking the carbohydrate-rich Fc portion of the immunoglobulin, $F(ab)_1$ fragments were prepared. The $F(ab)_1$ fragments transported as well as the whole antibody. When tested in competition with WGA, the transport of the $F(ab)_1$ was blocked, as before. This suggests that the lectin competes directly for the antibody binding sites. These studies indicate that the uptake and transport of the antibody is mediated by a subset of the wheat germ agglutinin binding glycoconjugates.

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381.6 PHOSPHORYLATION AND CALCIUM-ACTIVATED PROTEOLYSIS OF NEUROFILAMENT PROTEINS IN THE SQUID GIANT NEURON

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The posttranslational modifications of neurofilament proteins were studied in the squid giant neuron. The ability of endogenous phosphorylation and calcium activated proteolysis to modify neurofilament proteins was studied by 1.) identifying the products formed after endogenous phosphorylation and calcium activated proteolysis of the neurofilament proteins and 2.) by examining the cell bodies and axoplasm for modified neurofilament proteins. Phosphorylation was initiated by adding 10-20 μCi ($\gamma^{32}P$) ATP to homogenized axoplasm or cell bodies. The proteins were separated by polyacrylamide gel electrophoresis (PAGE). Gels were then dried and autoradiographed. To study calcium activated proteolysis 6 mM CaCl was added to homogenized axoplasm or cell bodies. The reaction was stopped with 2% SDS, 5% BME and the proteins were separated by PAGE and then stained with Coomassie Blue or silver.

When axoplasm is incubated in the presence of ($\gamma^{32}P$) ATP most of the phosphate is incorporated into two neurofilament proteins: one at 220 kilodaltons (K) (NF-220), and a high molecular weight neurofilament protein (NF-HMW). Endogenous calcium activated proteolysis cleaves the NF-220 more rapidly than the NF-HMW. The initial proteolysis of the NF-220 produces a pelletable fragment at 100 K and two soluble fragments: one at 160 K and a second at 110 K. The latter contains most of the $^{32}P$. The cell bodies of the giant neuron, unlike the axoplasm, contain no detectable NF-220. The cell bodies do, however, contain a number of proteins that both bind to a monoclonal anti-intermediate filament (IF) antibody, and are sensitive to endogenous calcium activated proteolysis. This IF antibody detects all the axoplasmic proteins that copurify with neurofilaments, i.e., in the order of their IF staining intensity: a 60 K, 65 K, 220 K, and a 74 K protein. In the cell bodies the IF antibody labels, in order of their staining intensity: a 65 K, 60 K, 74 K and a 180 K protein. The presence of different major (65 K instead of 60 K) and minor (an unphosphorylated 180 K instead of a phosphorylated 220 K) intermediate filament proteins in the cell bodies versus the axoplasm suggest that the intermediate filament (neurofilament) proteins synthesized in the cell bodies may be enzymatically modified before being transported into the axon.

381.7 DIFFERENCES IN THE AXONAL TRANSPORT OF CYTOPLASMIC MATRIX PROTEINS IN THE SOMATIC-MOTOR AND PARASYMPATHETIC OCULOMOTOR AXONS. P. Paggi and R.J. Lasek. Dept. of Cell. and Dev. Biol., University "La Sapienza", 00185 Rome, Italy, and Dept. of Dev. Genetics and Anatomy, C.W.R. University, Cleveland, Ohio 44106, U.S.A.

Studies of the axonal transport of radiolabelled cytoskeletal proteins can provide information about the transport characteristics of the cytoskeleton in axons (Lasek, R.J., J. Cell Biol. 99:212s, 1984). We compared the kinetics of transport of the cytoplasmic matrix proteins in the two functionally distinct axon populations that are part of the oculomotor system in chickens. The oculomotor nerve contains two populations of axons: somatic-motor (M) axons and parasympathetic (P) axons.

Three-old-week chickens received injections of $^{35}S$-methionine in the cerebral aqueduct and were sacrificed from 1 to 93 days after injection. Labeled proteins were analyzed in segments of the oculomotor nerve, which contains the M and P fibres; in the ciliary ganglion, where the P terminals are located; and in the somatic-motor branches of the oculomotor nerve where M axons course. The radiolabeled proteins were subjected to one and two-dimensional SDS-PAGE and fluorography, and were quantified by counting the excised bands.

As in other neurons, the cytoplasmic matrix proteins were transported at two different rates corresponding either to the slow component (SCa) which is defined by the neurofilament proteins (NFP) and tubulin or to the slow component (SCb) which is defined by actin, clathrin and other matrix proteins. The rate of the SCa proteins differed in M and P axons. NFP moved three times faster in M axons than in P axons. In M axons most of the tubulin moved coherently with the NFP and only a small fraction of the tubulin moved ahead of the neurofilaments. In P axons tubulin moved with NFP in SCa but a large fraction of the tubulin also moved with the proteins of SCb. These results suggest that the dynamic structural relationships between microtubules and neurofilaments in somatic-motor axons differ from those in the parasympathetic neurons of the oculomotor system.

381.8 RETROGRADE AXONAL TRANSPORT IN MOTOR AND SENSORY AXONS OF RAT SCIATIC NERVE. D.J. Fink, D. Purkiss,* and M. Mata. Neurology Research Laboratory, University of Michigan and VA Medical Center, Ann Arbor, MI 48105

We have previously used $^{3}H$ N-succinimidyl propionate ($^{3}H$ N-SP), an in-vivo acylating agent, to label endogenous axonal proteins within the nerve in order to study their subsequent bidirectional transport. In sciatic nerve slow anterograde and slow retrograde transport were labeled, but rapid transport in either direction was not seen. In the neurosecretory neurons of the neurohypophysis however, accumulation of rapidly transported components could be detected in the nerve terminals at the pituitary. We undertook the current study to determine whether accumulation of rapidly retrogradely transported $^{3}H$ N-SP labeled proteins could be detected at the cell bodies of sensory and motor axons.

One microliter containing 67 microCuries of $^{3}H$ N-SP (50Ci/mmol) was injected subepineurially in the sciatic nerve of 200-225 gram Sprague Dawley rats. At times ranging from 6 hours to 28 days after injection the animals were sacrificed by intracardiac perfusion and the sciatic nerve and dorsal root ganglia processed for total and TCA precipitable counts. The spinal cord was sectioned in a cryostat and the ipsilateral and contralateral lumbar ventral horns sampled using the Palkovitz punch technique and processed for both total and TCA precipitable radioactivity.

The amount of counts appearing in the sensory neurons and motor neurons respectively were expressed relative to the total amount of counts remaining in the nerve. DRG neurons showed a biphasic accumulation of radioactivity, with early accumulation peaking at 24 hours after injection and a larger accumulation occurring over the subsequent week. Motor neurons showed the first accumulation over 7 days, and a second larger accumulation by 2 weeks after injection. The difference in time of accumulation corresponds to the difference in distance from the injection site to the cell body of the sensory and motor neurons respectively, suggesting that rapid and slow retrograde transport of endogenous proteins occurs in both neurons, at equivalent rates, and that each contains roughly the same proportion of transported material. Control nerves, ligated between the injection site the DRG, showed no accumulation of radioactivity in the DRG or the spinal cord.

Light microscopic autoradiography of both DRG and spinal cord at 1 day and 5 days after injection showed grains localized specifically within the neurons in both the DRG and the spinal cord. Samples of both the DRG and the spinal cord neurons were separated by SDS gradient gel electrophoresis and fluorographed.

381.9 **AXONAL TRANSPORT AND COMPOSITION OF GANGLIOSIDES IN SENSORY AXONS OF RAT SCIATIC NERVE.** G.J. Harry,*, A.D Toews* and P. Morell. Biol. Sci. Res. Ctr. & Dept. of Biochem., Univ. of N.C., Chapel Hill, NC 27514.

**The synthesis and axonal transport of gangliosides and glycoproteins in sensory neurons of rat sciatic nerve were examined at various times following injection of [$^3$H]glucosamine into the L5 dorsal root ganglion. Incorporation of labeled precursor into these glycoconjugates at the ganglion continued for 6-12 hours following injection. Outflow patterns for labeled gangliosides were compared to those for glycoproteins at times from 3 to 24 hrs after precursor injection. The crest of transported radioactivity for both of these classes of glycoconjugates moved down the sciatic nerve at a rapid rate of approximately 360 mm/day. Although radioactivity in glycoproteins in the ganglion was 15-20 fold greater than that in gangliosides, levels of transported glycoprotein radioactivity in the nerve was only about 10 fold greater than gangliosides. This suggests a possible preferential commitment to transport for gangliosides, relative to glycoproteins.**

**The composition of gangliosides transported down the axons (radioactive gangliosides accumulating at a ligature on the nerve) differed somewhat from that synthesized in the ganglion. $GM_3$ was preferentially retained in the ganglion (presumably in the neuronal perikarya), while the tri- and tetrasialogangliosides were preferentially exported. The distribution of radioactivity among individual ganglioside species synthesized locally in the nerve (following intraneural injection of [$^3$H]glucosamine) differed substantially from the pattern of gangliosides synthesized in the ganglion and from that committed to transport.** **This work was supported by USPHS grants NS11615 and ES01104.**

381.10 EXAMINATION OF THE REDISTRIBUTION OF LYSOSOMES AND LIPOFUSCIN GRANULES FOLLOWING DISRUPTION OF NORMAL MICROTUBULE FUNCTION. V.J. Roberts, M.C. Bundman, G.O. Ivy, C. Gorenstein. Department of Pharmacology and Center for the Neurobiology of Learning and Memory, University of California, Irvine 92717.

Under normal conditions, lysosomes and lipofuscin granules are localized in neuronal cell bodies. We have previously shown that injections of a mitotic inhibitor, colchicine, into the lateral cerebral ventricle of the rat induced a paradoxical translocation of these organelles from the cell somata of neurons into the dendrites (Gorenstein et al., J. Neurosci., in press; Gorenstein and Ribak, J. Neurosci., in press).

The thiol proteinase inhibitor, leupeptin, has been shown to cause an increased concentration of neuronal lipofuscin-like granules (Ivy et al., Science 226 1984), a condition resembling the aged brain. Colchicine treatment (100 µg i.c.v) caused a redistribution of these granules similar to that seen with lysosomes. In order to characterize this modulation further, the time course of the redistribution of lysosomes and lipofuscin granules following colchicine treatment was examined quantitatively at the electron microscope level.

Microtubules must be able to polymerize and depolymerize for normal cellular function. The effect of taxol (a mitotic inhibitor with microtubule stabilizing properties) on the distribution of lysosomes was also examined. Taxol (90ug) was injected into the lateral cerebral ventricle of rats. Cellular death occurred in parts of the cerebellum 5 days following treatment. However, while both taxol and colchicine disrupt normal microtubule function, only colchicine affected the normal cellular distribution of lysosomes. We speculate that this redistribution may be due to a disruption of microtubules which normally sequester lysosomes within the cell body. To account for the specific movement of lysosomes into dendrites once microtubules are disrupted, we also propose that lysosomes are coupled to a unique transport system which specifically directs these organelles into dendrites.

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381.11 A METHOD FOR THE ELECTROPHYSIOLOGIC STUDY OF ENZYME DISSOCIATED AXONS IN THE RAT SCIATIC NERVE. R. Rumpf, T. Spagnolia, W.J. Levy, D.H. York. University of Missouri Health Science Center, Columbia, Missouri 65212

Existing methods for studying axons have involved whole nerve preparations, axons teased from excised nerves, or tissue culture. To complement these it would be advantageous to study the normal and pathologic function of isolated, separate axons in vivo, still in continuity with the CNS and end organ. To achieve this the surgically isolated sciatic nerves of pentobarbital anesthetized, adult male rats were passed through a glass-bottom Silastic$^R$ chamber designed for controlled temperature, pH and flow rate superfusion with physiologic saline solutions and enzyme dissociation solutions. This chamber was mounted on the stage of an inverted phase contrast microscope. The process of axon dissociation by superfusion with $Ca^{++}$-free Hank's Balanced Salt Solution (HBSS) followed by 1% Collagenase in full complement HBSS was monitored and photographed with the high-power inverted microscope. This process was enhanced by dissecting the epineurium off before dissociation, and by including thrombin (100 units/ml) and vasopressin ($1 \times 10^{-8}$ M) in the dissociation solutions to reduce bleeding. Using this technique 1 cm of sciatic nerve could be reliably dissociated into free, separate axons substantially free of extracellular matrix in 2 to 4 hours at 37° C. EMG recordings from the lumbrical muscles of the foot and peripheral nerve recordings from the tibial nerve obtained before and after enzyme dissociation were not dramatically different. Osmium tetroxide (2% in phosphate buffer) staining of dissociated axons showed no obvious morphologic damage. Single axon action potentials were recorded with extracellular pipette microelectrodes (2 M NaCl, tip resistance 2 - 10 Mega Ohms) under mineral oil. Action potential propagation was thus studied under conditions of reversible cold and lidocaine blocks, selective cation exclusion and anoxia. Axons separated by this technique were also found to maintain axoplasmic transport (Spagnolia, Rumpf, Levy and York, NEUROSCIENCE ABSTRACT, October, 1985). With this model action potential properties were studied up to 12 hours. This model provides a large percentage of usable axons which do not show observable damage and may be useful for the study of normal and pathologic peripheral nerve function.

381.12 THE MAUTHNER NEURON AS AN IN VITRO VERTEBRATE MODEL TO STUDY AXOPLASMIC TRANSPORT. Edward Koenig, Div. of Neurobioilogy, Dept. of Physiology, SUNY at Buffalo, Buffalo, NY 14214.

The two principal in vitro models that have been used to study organelle movement in axons are from invertebrates; namely, the giant axons of the squid (Allen, et al., 1982, Science 218:1127), and of the lobster (Forman, et al., 1982, Soc. Neurosci. Abstr. 8:827). The squid preparation consists of expressed axoplasm, lacking its rim of ectoplasm, while the lobster preparation includes its sheath and is permeabilized by a detergent. A potentially good vertebrate model is the large axon of the Mauthner cell (M-cell) from goldfish, which ranges from 40-80 µm in diameter (Funch, et al., 1981, Neurosci. Lett. 27:159).

The M-cell axon in a native state can be translated out of its myelin sheath with a pair of microtweezers under Fluorinert, a fluorinated aliphatic liquid. Movements of organelles can be visualized with time-lapse video phase-contrast microscopy. However, organelle movement activity is critically dependent on the composition of the solution in which the brain and spinal cord are suspended before axons are exteriorized. This is apparently due to the fact that some extracellular fluid "coats" the axon when it is translated out of its in situ location. Neither a standard fish physiological salt solution (e.g., Cortland), nor a number of potassium-containing artificial "internal" media yields discernible movement activity in isolated axoplasm. However, a betaine based medium (0.28M) with Mg (2mM), EGTA (1mM), glucose (5mM), Mes (20mM), beta-alanine (50mM) and spermidine (1mM) yields a preparation with spontaneous movements of organelles that continue for 30-45 minutes. Axons isolated in this medium with reduced spermidine (0.1mM) also reveal spontaneous organelle movements when supplemented with ATP (2mM), dithiothreitol (0.2mM), polyvinylpyrrolidone (3%) and sodium pyrophosphate (5mM); however, the level of movement activity is reduced compared to those isolated under Fluorinert. The basis for cessation of movement activity after a period of in vitro isolation has not been investigated, and it is not clear whether the basis is the same when axons are isolated under Fluorinert or bathed by an artificial medium. Preliminary measurements of saltation rates of several classes of organelles range from 0.07 to 1.58 µm/sec. at 20 degrees C.

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381.13 FAST AXONAL TRANSPORT IN SQUID AXOPLASM IS MEDIATED BY CROSS BRIDGES BETWEEN MICROTUBULES AND VESICLES. R.H. MILLER* and R.J. LASEK. Dept. of Anatomy, Case Western Reserve Univ., Cleveland, Oh. 44106.

The recent demonstration that individual microtubules at the periphery of axoplasm extruded from the squid giant axon will support vesicle transport clearly demonstrates that microtubules can provide the linear substrate for vesicle transport in axons (Schnapp, et.al. Cell 40:455,1985).

We have examined the detailed morphological relationships between actively moving vesicles and their substrate microtubules at the periphery of extruded axoplasm, using thin sections and transmission electron microscopy. Vesicle transport was focally interrupted by the application of a short term cold block, and anterogradely and retrogradely moving vesicles collected on either side of the block. This approach increased the number of vesicles available for analysis. When the cold block was released by rewarming the preparation, the accumulated vesicles began moving again in their former direction, as shown by AVEC-DIC microscopy. The region of axoplasm containing large numbers of actively moving vesicles was then fixed and processed for TEM.

Longitudinal sections through the axoplasm showed that at the periphery the axoplasm expanded and individual microtubules became separated from the other cytomatrix components, allowing detailed analysis of the vesicle-microtuble interactions. In this region, both the anterogradely and retrogradely moving vesicles were preferentially associated with the individual microtubules, indicating that the actively moving vesicles have a higher affinity for the microtubules than for other cytomatrix components, (i.e.the neurofilaments). Detailed analyses show that both the anterogradely and the retrogradely moving vesicles were attached to their substrate microtuble by 16-18nM crossbridges.

This observation supports the crossbridge model for vesicle transport. We propose that the 16-18nM crossbridges connecting the vesicles to microtubules are the ATPase bearing structures that generate the force for vesicle translocation.

381.14 INFLUENCE OF TRANSLOCATION TRACK ON THE MOTION OF INTRA-AXONALLY TRANSPORTED ORGANELLES IN HUMAN NERVE. M.P. Lynn,* A.C. Breuer and M.B. Atkinson.* Cleveland Clinic Foundation, Cleveland, OH 44106.

The mechanism by which organelles are transported bidirectionally in axoplasm is still unknown; however, evidence of a key role for microtubules has been mounting. We have observed common or shared tracks within the axoplasm of human nerves along which multiple organelles of varying size and shape are bidirectionally transported. To investigate this phenomenon, we obtained human nerves from patients undergoing limb amputation or en-block resection for tumor removal. The motion of organelles traveling anterogradely and retrogradely was visualized by video-enhanced differential interference contrast optics and analyzed with the aid of computer image processing techniques.

Speeds of translocating organelles were determined at eight to 16 translocation points along a path or "track". Each translocation speed was then plotted against its corresponding position on the tract to develop a "speed/position diagram". In axonal regions containing more than one common tract some tracks exhibited greater activity (number of organelles/unit time) than others. However, the average mean translocation speed of organelles on a common track was not related to the activity of the track.

A range of mean translocation speeds was evident on each common track in an axon. Regardless of mean organelle speed or direction of motion, organelles sharing a common track exhibited similar patterns of "speeding up" and "slowing down" relative to position along the track. Speed/position data for organelles translocating in the local axonal region of a common track showed no unique patterns (not different from a uniform distribution, $p < 0.05$). The unique speed/position patterns exhibited by common tracks were not necessarily related to the patterns of other tracks in the immediate vicinity (distance between tracks $< 0.50$ μm). These findings suggest that 1) there are "common tracks" shared by organelles moving retrogradely and anterogradely; 2) both the organelles and the "track" associated with its translocation play a role in the resultant motion of that organelle; 3) the influence exerted by a common track on the motion of an organelle results in a pattern of speed changes related to position along the track.

381.15 ANTEROGRADE AND RETROGRADE AXOPLASMIC TRANSPORT HAVE THE SAME VELOCITY. E.J. Muñoz-Martínez & W. Ejido* (SPON: E. Stefani). Dept. Physiology, Biophysics and Neurosciences, CINVESTAV. A.P. 14-740. 07000-México, D.F.

Previous determinations of the retrograde velocity of endogenous proteins intraaxonally transported involves considerable uncertainties. In general, it has been estimated that the velocity of retrograde transport is about half of that for anterogradely transported proteins. The experiments reported here indicate that the velocity of fast axonal transport is unique independently of the transport direction. Cats were anaesthetized (pentobarbitone 35 mg/Kg I.P.) to expose the seventh lumbar spinal ganglia bilaterally. Each ganglia received 10 μl of $^{3}$H-Leucine and anterograde transport was allowed to proceede for 10 hs after injection. Thereafter, two ligations were made in the sciatic nerves at 3.5 and 7 cm from the ganglia and transport was allowed to proceed in the isolated portion of the nerve for variable intervals (0.5-10 hs) before killing the animals.
The isolated portions of the sciatic nerves were removed and sectioned in 5 mm long pieces to determine radioactivity by scintillation counting. It was found that in the 5 mm segment just distal to the proximal ligation, the radioactive label initially decreased indicating deplection due to anterograde transport, but subsequently increased indicating proximal accumulation of proteins previously accumulated in the most distal 5 mm segment of the isolated portion of the nerves. Accumulation in the proximal segments started 2-2.5 hs after the simultaneous ligations. Thus, assuming that retrograde transport started immediately after ligation, the velocity of this transport was 14 to 17.7 mm/h which closely coincide to that found for anterograde transport. It is concluded that both anterograde and retrograde transport may use the same transport system.

382.1 TOPOGRAPHICAL ORGANIZATION OF THE FACIAL NUCLEUS IN THE RABBIT. Ronald H. Baisden, Michael L. Woodruff, Duane C. Baker*, Dennis L. Whittington and Amy E. Benson*. Department of Anatomy, Laboratory for Neurobehavioral Science, Quillen-Dishner College of Medicine, East Tennessee State University, Johnson City, TN 37614.

The topographical representation of the branches of the facial nerve in the motor nucleus of CN VII was studied in 33 rabbits following retrograde transport of HRP. In cross section, the facial nuclear complex appears to be formed from a series of four subnuclei. A large medial subnucleus (MSN) is separated from a slightly smaller lateral subnucleus (LSN) by a slender intermediate subnucleus (ISN). A small accumulation of cells composing the superior subnucleus (SSN) is found dorsal to the LSN. Cells which give rise to the various branches, however, are not confined to a particular subnucleus but appear to be distributed in topographical fashion throughout the complex. The cells which give rise to the mandibular branch are located predominately in the LSN and ISN, however some cells are found in the SSN. The buccal branch innervating the anterior muscles of the face and lips split into a superior and inferior division. They both arise from a common population of cells located in the LSN and ISN. The zygomatic branch to the upper face below the orbit is derived from cells which cap the facial nuclear complex with the majority found in the SSN, but which extend over and into the medial aspect of the MSN. In contrast, the auriculopalpebral branch to the eyelids comes from a large mass of cells in the MSN as well as a dorsal group that extends over into the SSN. This same pattern is observed in the organization of the cells that give rise to the rostral auricular branch to the anterior ear. The posterior auricular branch arises exclusively from cells of the MSN, as does the cervical branch, although in this case labeled cells are confined to the ventrolateral portion of the MSN. The nerves to the stylohyoid, the platysma and the digastric are all derived from cells which primarily reside in the ISN, although the digastric, which in the rabbit consists only of an anterior belly, also receives a substantial innervation from cells located in the posterior medial portion of the trigeminal motor nucleus as well as a string of cells which connect with a cluster of cells found outside of, and dorsal to, the facial complex proper. No attempt was made in the present study to determine the origin of the nerve to the stapedius muscle. These studies demonstrate that the facial nucleus is organized in a topographic pattern reflecting the anatomy of the facial musculature. The results are consistent with reports of facial nucleus organization in other mammalian species.

382.2 SYMPATHETIC INNERVATION OF THE TYMPANIC PLEXUS IN MACACA FASCICULARIS. PJ Gannon* and AR Eden. Dept of Otolaryngology, Mt. Sinai School of Medicine of the City University of New York, NY 10029.

The extent of the sympathetic innervation to the tympanic plexus is largely unknown. This study was designed to analyze the number of fibers in the tympanic plexus originating from the superior cervical ganglion (SCG) using an E.M. ultrastructural degeneration procedure. In humans, the tympanic plexus consists of efferent and afferent nerve fibers from the tympanic branch of the glossopharyngeal nerve, the auricular branch of the vagus, and sympathetic fibers from the external carotid nerve plexus via the caroticotympanic nerves. A similar arrangement is found in old world monkeys such as macaques.

Animals were unilaterally sympathectomized by surgical excision of the SCG and 2-3mm of the superior nerve trunk to ensure removal of all ganglion cells. The distal stump was cauterized and the animals allowed to survive 20 and 40 days. Excised SCG were wax embedded and sectioned to observe the extent of the ganglion cells. Animals were fixed transcardially, then the middle ear entered and the nerves postfixed in situ with osium tetroxide. The nerves were dissected out from various points in the tympanic plexus and processed for E.M. whole nerve transverse sectioning. Total counts (in duplicate) of degenerating myelinated and unmyelinated fibers were made from electron micrograph photomontages at a magnification of 3000x.

Analysis of Nissl-stained 5um sections of excised SCG indicated that the sympathectomy was complete and that all ascending fibers had been transected. Quantitation of degenerating fibers in tympanic plexus nerves showed a consistent pattern. Caroticotympanic nerves entering the plexus showed 35-44% myelinated and 17-20% unmyelinated nerve fiber degeneration, while the tympanic nerve showed 34-46% myelinated and 18-28% unmyelinated fiber degeneration. A hypotympanic branch of the tympanic nerve was present in one animal and was analyzed separately.

Future studies will determine the specific innervation of organs such as the parotid gland and inner ear via the tympanic plexus.

382.3 FLUORESCEIN PENETRATES MIDLINE BRAIN STRUCTURES OF FEMALE RAT BRAINS TO A GREATER EXTENT THAN MALE BRAINS. Leonard Koda, Joe L. Martinez, Jr.*, and Floyd Bloom. Scripps Clinic and Research Foundation, La Jolla, CA 92037 and *Dept of Psychology, Univ of California, Berkeley, CA 94720.

Hormonal substances likely access the brain through the blood brain barrier, through the CSF via the choroid plexus, or through the circumventricular organs. In our attempt to identify entrance sites of peptides into brain we noticed greater penetration of fluorecein into female as compared to male rats.

In this study female and male (150-200 g) Charles River rats were anesthetized with pentobarbital. The abdominal cavity was opened below the diaphragm. A 22 ga needle was placed into the heart through the diaphragm and the rat was injected with 2% sodium fluorescein solution (.4ml/100g). Five m later the descending aorta was clamped and the rats were transcardially perfused with sal. For fluorescence photomicroscopy, 4% depolymerized paraformaldehyde in phosphate buffer was substitute for sal. Brain parts (frontal cortex, cerebellar cortex, rest of brain) were homogenized in 5 vol phosphate buffer. Samples were microcentriguted (Eppendorf) for 1 m. A 0.15 aliquot of supernatent was diluted with 1.35 ml phosphate buffer and measured in a fluorometer. There were no differences between the amount of fluorescein measured between males and females in cortex or cerebellum. However, females had significantly ($p = .002$) more fluorescein (1.73 + .14 nm/wet wt) than males (1.07 + nm/wet wt) measured in the rest of brain. This same relationship was observed when ovariectomized and castrated rats were examined. Photomicroscopy revealed intense fluorescence in the circumventricular organs and adjacent areas. Both the area postrema and nucleus tractus solitarius were fluorescent as well as the median eminence, paraventricular and arcuate nucleus, and the organum vasculosum and the suprachiasmatic area. The choroid plexus was fluorescent, but in contrast to the circumventricular organs, the adjacent areas did not fluoresce.

We conclude the fluorescein enters the female rat brain more readily than the male brain. This increased penetration is not associated with the blood brain barrier as cortex and cerebellum did not increased fluorescence. Rather it appears that the circumventricular organs and associated brain nuclei are primary sites of uptake. Further this sex difference is not maintained by sex hormones as gonadectomy did not alter fluorescein penetration. (Supported by ONR N00014-83-K-0408 and NIAAA 06420).

382.4 DESCENDING PROJECTIONS FROM THE MEDIAL VERSUS LATERAL PREFRONTAL CORTICES OF THE RAT. S.G.P. Hardy. Dept. of Anat., Univ. of Miss. Med. Ctr., Jackson, MS 39216.

In recent years there has been a resurgence of interest in the prefrontal cortex (PFC) and its connectivity. In the rat, the PFC is parcellated into medial and lateral subdivisions. Few attempts have been made to compare the connectivity of these subdivisions throughout the brainstem. In this study, using a variety of HRP techniques, it was determined that there are considerable differences in the trajectory of descending axons originating from these cortical subdivisions. Some of these differences are as follows:

From the medial PFC: Axonal labeling in the THALAMUS was heaviest in the dorsolateral part of the mediodorsal (MD) nucleus, medial aspect of the ventral thalamic nucleus and in the parafascicular nucleus. In the MIDBRAIN, labeling was most pronounced in the deep layers of the superior colliculus and dorsolateral periaqueductal gray. In the PONS, labeling was primarily in the parabrachial nuclei and lateral pontine tegmentum, although some labeling was also observed in the paramedian pontine reticular formation. No labeled axons were observed descending into the medulla.

From the lateral PFC: Axonal labeling in the THALAMUS was heaviest in the medial part of the MD nucleus, lateral aspect of the ventral thalamic nucleus, nucleus reticularis and zona inserta. In the MIDBRAIN, labeling was heaviest in the nucleus cuneiformis, nucleus of Darkschewitsch, medial accessory nucleus of Bechterew and substantia nigra. In the PONS, labeling was present in the parabrachial nuclei and lateral pontine tegmentum. At more caudal levels of the pons, labeled axons were observed to pass ventrally through the lateral tegmentum. In the MEDULLA, the labeled axons passed into the vicinity of the facial nucleus, then decussated in the region of the nucleus raphe magnus. The axons then assumed a position in the dorsal lateral tegmentum, lying medial to the spinal trigeminal nucleus. At caudal levels of the medulla, labeled axons passed medially to the dorsal column nuclei, hypoglossal nuclei and vestibular nuclei. There was also slight labeling of the nucleus of the solitary tract.

Additional studies were performed, in which HRP was placed into various brainstem sites. The resulting retrograde cortical labeling supported the findings of this study.

In this study it has been demonstrated that the descending projections from the medial versus lateral PFC are distinctive throughout their course in the brainstem. (Generally, the projections from the medial PFC were to dorsomedial brainstem sites, whereas projections from the lateral PFC were to lateral brainstem sites.) This suggests that these PFC subdivisions may be functionally distinct. (Supported by NIH grant BRS2S07-RR05386).

382.5 THE SO-CALLED "INITIAL" TYPE OF THE NEOCORTEX: RELATION TO CETACEAN BRAIN ORGANIZATION. I. Glezer*, M.S. Jacobs and P.J. Morgane (SPON: F.M. Liebman). CUNY Medical School, New York, NY 10031; Dept. Pathobiology, NYU Dental Center, New York, NY 10010; Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

The idea of the existence of the "archetypal" neocortical plate originates not in the field of neuroanatomy, but in comparative anatomy. All paleontological and comparative anatomical data confirm that the most primitive of extant living placental mammals are the Insectivora. Ancient Paleocenic representatives of this group are considered as ancestral to all placental mammals. From this notion evolved a concept that was intensively developed by Filimonoff and his school (Preobrashenskaja, N. et al., Z. Mikr.-Anat. Forsch., 87:490, 1973). This concept reiterates that if insectivores are the most primitive and closest to the ancestral stock then their brain and, especially, cortex may represent the "archetype" that then developed into specialized cortical types in different Orders of mammals. The main features of the archetypal neocortex are: (1) absence or weak representation of layer IV, (2) intense staining of densely packed cells in layer II, (3)hypertrophy of layer I which is extremely wide and contains abundant tangential axon branches as well as ramifications of apical dendrites of pyramidal cells of layers II, V and VI, and (4) overall slight development of the associative zones of the neocortex and predominance of the primary functional zones. This kind of cortex was found in some of the basal Insectivora (Valverde, F., Ch. 13, In: Ramon y Cajal's Contribution to Neuroscience. Elsevier, 1983). More recently in the dolphin brain (Morgane, P.J. et al., Evolutionary morphology of the dolphin brain, In: Conference on Dolphin..., Erlbaum, In press, 1985), many features have been found resembling those present in the hedgehog brain. Comparative anatomical classification of Cetacea disconnect this group from Insectivora at the time whales went to water. Hence, it is uncertain whether the so-called primitive features of the cetacean cortex are ancestral or originated as a result of the highly specialized aquatic evolution. We can contemplate two types of brain phylogeny. In the first or terrestrial type the emphasis was on diversity of the primary neocortical areas and on the prevalent growth of the non-specific (associative) zones between the primary zones. In the second type of evolution, as may be represented by purely aquatic Cetacea, the emphasis was on the unification of what might be initially diverse modules of the neocortex. The main direction of this type of evolution was in multiplication of the unified module which may have resulted in the expanded but architectonically "monotonous" cortex of dolphins. (Supported by NSF Grant BNS 84-14523).

382.6 A GOLGI STUDY OF NEURONAL COMPONENTS IN CONVEXITY NEOCORTEX OF DOLPHIN BRAIN. P.J. Morgane, M.S. Jacobs, I. Glezer* and A. Galaburda. Worcester Foundation for Expt. Biol., Shrewsbury, MA 01545; New York University Dental Center, New York, NY 10010; City College, New York, NY 10031 and Dept. of Neurol., Harvard Med. School, Boston, MA 02215.

Among extant insectivores, the hedgehog (Erinaceus europaeus) is regarded as perhaps the most direct modern descendant of the primitive placentals (Romer, 1974). It has been termed a "survivor of the Paleocene" (Romer, 1949). It has retained, apparently with minor variations, basic characteristics of its primitive insectivore ancestors. Hence, it is a reference for the study of what might be considered a prototype of neocortical organization and a valid, though not unique, model of neocortical phylogenetic development. In the present study we have compared some cytoarchitectural and morphological details in the hedgehog's auditory and visual neocortical areas with those in the cerebral cortex of the bottlenose dolphin. The aim is to define types of neurons that might be present in these cortices in relation to homologous types described in other species. Neurons showing a predominantly subpial dendritic arborization, i.e., "extraverted neurons" dominate in layer II of dolphin cortex and are mostly medium sized pyramids and polymorphous neurons. They occur over the entire convexity cortex in both dolphin and hedgehog brains. Since the extraverted neurons resemble closely those of the superficial cell condensations of the first and second growth rings of the neocortex, i.e., periallocortex and proisocortex, they are considered to represent an ancient type of cortical neuron. In most mammalian cerebral cortices this type is preserved only in the primitive allocortices. In dolphin layer II we see a range of neurons from moderately extraverted to well-balanced (approximately equal apical/basal dendritic systems) small pyramids. The functional significance of dendritic extraversion is that this type of protoneocortical organization represents axodendritic contact through the zonal layer which is a feature of organization in the allocortices. Golgi material in the dolphin shows the presence of long radiator (reticular) type stellate cells at many levels in the cortex and several varieties of giant stellate cells similar to those described in the hedgehog brain by Sanides (1972). There is no trace of a hypergranular cortex anywhere in the dolphin convexity cerebral cortex, with layer IV being absent. Convexity cortex in dolphin is dominated by transitional forms of neurons varying between pyramids, spindles and stars. Study of the dolphin cortex as compared to that of the hedgehog cortex reveals many striking similarities. At the level of resolution provided by the Golgi method, our findings suggest in both species a stage of cortical evolution in which a general allocortical plan of neuronal organization is dominant (Supported by NSF Grant BNS 84-14523).

382.7 HIPPOCAMPAL FORMATION OF DOLPHIN: A MORPHOMETRIC GOLGI STUDY. M.S. Jacobs, I. Glezer* and P.J. Morgane. Dept. Pathobiology, NYU Dental Center, New York, NY 10010; CUNY Medical School, New York, NY 10031; Worcester Foundation Exp. Biology, Shrewsbury, MA 01545.

Since describing the cytoarchitectonic features of the archicortex in Tursiops truncatus (Jacobs et al., Brain Res. Bull., 4:Suppl.1, 1979), we have recently applied a modified Golgi-Kopsch method to a study of the cellular morphology of the hippocampal formation (HF). Well impregnated cells in the dentate gyrus (DG), hippocampal fields CA4, CA3, CA2, CA1 as well as the subiculum (Sb) have been examined morphometrically. In the paucicellular molecular layer (ML) of the DG are found cells resembling displaced granule cells (GC) as well as stellate neurons. The densely packed granular layer (GL) contains predominantly small (16.1um x 12.3um) oval GC with moderately spined apical dendritic branches extending well into the ML. In addition, basket cells are found intermixed with typical GC in the layer. The hilar region contains scattered medium sized polymorphic basket cells (21.0um x 14.3um) as well as medium sized non-aligned pyramidal cells (21.9um x 13.6um), the latter having dendrites that are more densely spined than GC. The pyramids of CA4 are scattered, small (18.0um x 12.0um) and have well developed apical and moderately developed basal dendrites. Continuous with the pyramids of CA4 appears the narrow cell band of the stratum pyramidale (SP) of CA3. Most CA3 pyramids lack a typical pyramidal shape, are medium to large sized (28.6um x 15.4um) and have moderately spined apical and basal dendrites. Cells from the SP scatter into the stratum oriens (SO). In counterstained Golgi material there is a progressive widening and increase in density of the SP from CA3 to CA1. In CA2 the SP separates into a denser, patchy superficial sublayer and a less dense, more homogeneous deep sublayer. The pyramids of CA2 are smaller (21.2um x 14.9um) than in CA3 and do not scatter as much into the SO. In CA1 the pyramidal cells are medium in size (25.7um x 13.7um) and are characterized by more densely spined and more heavily branched apical and basal dendrites than in other hippocampal fields. In counterstained Golgi sections the SP is more densely cellular than CA2 and continues to show the same sublamination seen in CA2. Extending from CA1 to Sb the SP widens but becomes less dense. The Sb is characterized by distinctly different populations of pyramids at different levels of the cortex. The contrast is sharp between club shaped deep pyramids with long apical dendrites branching near the plexiform layer and basal dendrites contributing to a horizontal plexus, and more typical superficial pyramids with short, widely branching apical dendrites and obliquely oriented basal dendrites. This Golgi pattern enables the border between the Sb and CA1 to be identified. Overall, the HF of the dolphin has many features similar to those in other species, including primitive extant insectivores. This reflects the generally conservative character of the HF throughout phylogeny. (Supported by NSF Grant BNS 84-14523).

382.8 ABSENCE OF SEXUAL DIMORPHISM OF THE CORPUS CALLOSUM IN SCHIZOPHRENIA: A MAGNETIC RESONANCE IMAGING STUDY. H.A. Nasrallah, N.C. Andreasen, S.C. Olson*, J.A. Coffman*, C.E. Coffman*, V.D. Dunn*, J.C. Ehrhardt*. Departments of Psychiatry and Radiology, Ohio State University, Columbus, OH and The University of Iowa, Iowa City, IA.

A post-mortem study (DeLacoste-Utamsing and Holloway, 1982) reported that right handed (RH) males and females show gender-related morphological differences in the corpus callosum, with a larger splenium in females. This difference was attributed to the developmental differentiation of the right cerebral hemisphere in males for visuo-spatial tasks, and the reduction in posterior (splenial) interhemispheric callosal fibers. We present the first in vivo study of callosal sexual dimorphism in both normal subjects and schizophrenic patients, using magnetic resonance imaging (MRI).

Volunteer RH males (N=11), RH females (N=10), LH males (N=10) and LH females (N=10) were recruited into a MRI brain study of callosal morphology. In addition, RH male schizophrenics (N=23), RH female schizophrenics (N=10) and LH male schizophrenics N=5) consented to participate in the protocol. Several studies have suggested abnormalities in callosal structure and function in schizophrenia (Nasrallah et al, 1982).

MRI scans were obtained with a Picker .5 Tesla superconducting magnet. The midline saggital (inversion-recovery) view was used for this study. The corpus callosum was divided into four quartiles. The percentage area of the posterior quartile relative to the entire corpus callosum area was measured in all subjects and the means of the groups (by gender, handedness and diagnosis) were statistically compared.

The posterior quartile proportion of the callosal area was significantly larger in normal RH females (36.3%) compared to normal RH males (33.4%) but no difference emerged between schizophrenic RH females (32.8%) and schizophrenic RH males (34.4%). In fact, a trend for the opposite pattern was noted in the schizophrenic group. Finally, LH males did not differ from females regardless of handedness and female schizophrenics had a significantly smaller posterior quartile than female controls.

The results replicate the post-mortem findings of sexual dimorphism in the corpus callosum, and suggest that this sexual dimorphism is absent in schizophrenic patients. The significant decrease of the posterior callosal quartile in female schizophrenics is suggestive of a possible "masculinization" in the brain of female schizophrenics, and perhaps a different pattern of cerebral hemisphere organization compared to normal females.

382.9 CONVERGENCE, DIVERGENCE AND NEURONAL NUMBER IN PERIPHERAL SYMPATHETIC PATHWAYS OF MAMMALS OF VARYING SIZE. W. Snider*, D. Purves, E. Rubin, and J. Lichtman. Departments of Neurology and Anatomy and Neurobiology, Washington University School of Medicine, 660 S. Euclid Ave., St. Louis, MO 63110.

The largest mammalian species are at least 10 million times the size of the smallest. This enormous range in animal size raises a fundamental problem for nervous system function: how do larger animals maintain adequate control of peripheral structures that are many times more massive and extensive than the homologous structures in smaller animals? In order to study this question we have explored several quantitative aspects of the organization of the peripheral sympathetic nervous system of five mammals (mouse, hamster, rat, guinea pig, and rabbit). We have chosen the superior cervical ganglion and its pre-ganglionic supply in the spinal cord as a relatively simple neural pathway in which the numbers of pre- and post-synaptic neurons, the numbers of axons innervating each ganglion cell (convergence), and the number of ganglion cells innervated by each axon (divergence) can be assessed with reasonable accuracy.

The average adult weights of these species vary over approximately a 65-fold range. However, the number of superior cervical ganglion cells increases by only a factor of 4 between the smallest of these animals (mice; about 25 grams) and the largest rabbits; about 1700 grams); the number of spinal pre-ganglionic neurons that innervate the ganglion increases by only a factor of 2. Thus, the numbers of nerve cells in the sympathetic system do not increase in proportion to animal size.

It is obviously of interest to ask how larger animals might compensate for this relative deficiency in numbers of neurons. Several differences in the organization of this peripheral sympathetic pathway are apparent across these species. Ganglion cells increase in size from the smaller of these species to the larger and bear progressively more complex dendritic arbors. In addition, there is a progressive increase in the number of axons contacting each ganglion cell. The number of ganglion cells innervated by each pre-ganglionic neuron can be estimated by multiplying the degree of convergence by the ratio of ganglion cells to pre-ganglionic neurons. This estimate of preganglionic divergence also increases progressively across these species from approximately 60 in the mouse to approximately 400 in the rabbit.

We suggest that modulation of convergence and divergence in sympathetic ganglia allows this part of the nervous system to effectively activate homologous peripheral targets over a wide range of animal size. Preliminary results in a parasympathetic ganglion (the submandibular) suggest that some of these relationships hold for the autonomic nervous system in general.

382.10 THE CYTOARCHITECTURE OF THE CERVICAL AND THORACIC SPINAL CORD OF THE RABBIT. P.L. Vera*, T.W. Jarrell*, H.H. Ellenberger*, J.R. Haselton*, C.L. Haselton* and N. Schneiderman (SPON: W.H. Evoy). Psych. Dept., University of Miami, Coral Gables, Fl. 33124.

The cervical and thoracic cords of 5 adult and 2 neonatal rabbits were examined using the laminar scheme presented by Rexed (J. Comp. Neurol., 1954, 100, 297-380) for the spinal cord of the cat. Serial transverse or horizontal frozen sections (40, 60 or 80 um) were stained with cresyl violet, thionin or a modified Weil stain for myelin. Lightfield and darkfield illumination were used to examine the sections. Darkfield illumination was found to be particularly useful in determining the layers of the dorsal horn.

All ten laminae proposed by Rexed were identified in the spinal cord of the adult rabbit although they were easier to identify in the neonatal cord. Layers I through IV were similar to the cat, with the exception that layer IV appeared to extend down the medial border of the dorsal horn to a greater extent. The reticulation of layer V appeared to be more extensive than was reported for cats. It occupied a larger area dorsoventrally, and mediolaterally it extended for 1/2 to 2/3 of the dorsal horn.

Within layer VII, at the level of the second thoracic segment, the lateral horn (LH) appeared for the first time. The area of the LH was greatest at this level (11096 square microns) and it decreased caudally until the level of T6 (5396 square microns). Beyond T6, the LH became even further reduced and started disappearing so that it was not present in every section. Also, at the levels where LH was present (T2-T6), it appeared to be separated from the intermediate grey matter of lamina VII by intervening white matter. The separation was rarely complete so that often the top and bottom of the LH were connected with the intermediate grey matter. The separation was greatest at T2 (69 um) and it decreased caudally until it disappeared at T7, with the disappearance of the LH.

In the ventral horn, the cervical enlargement started at the caudal level of the third cervical segment, with the appearance of the phrenic nucleus, and it extended into the first thoracic segment.

Outside of the grey matter, a number of cells were found lateral to the lateral border of the dorsal horn in the area that Rexed called the lateral cervical nucleus. This aggregation of cells was more prominent in cervical segments 1-3. However, a number of scattered cells were present in the same location throughout the rest of the cervical and thoracic cord. Therefore, it might represent a similar nucleus to the lateral spinal nucleus described in the rat (Molander et al, 1984, J. Comp. Neurol., 1984, 230, 133-141).

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382.11 CONNECTIONS OF THE NUCLEUS OF THE SOLITARY TRACT (nTS) IN THE PIGEON. J.M. Wild, J.J.A. Arends* and H.P. Zeigler, Dept. of Anatomy, School of Medicine, University of Auckland, Auckland, New Zealand, and Biopsychology Program, Hunter College, CUNY, New York, N.Y. 10021.

A combination of autoradiographic and histochemical tracing procedures was used to identify the connections of the avian nTS. Evoked potentials to cervical vagal nerve stimulation were used to control the stereotaxic placement of large pressure injections of tritiated amino acids (proline/leucine) into nTS at various rostrocaudal levels. Confirming previous reports in mammals, terminal labelling was seen in the subjacent reticular formation, in the parabrachial nuclear complex (nPB), and in a periventricular region of the posterior hypothalamus. In contrast to mammalian findings no terminations were seen at telencephalic levels.

Iontophoretic and pressure injections of wheat germ agglutinin-horseradish peroxidase (WGA-HRP) into nTS provided anterograde data confirming these projections and produced retrograde labelling of neurons within nPB and the nucleus periventricularis magnocellularis (PVM) and the stratum cellulare externum of the hypothalamus. WGA-HRP injections into the hypothalamus or nPB produced retrograde labelling of nTS neurons, particularly those within subnuclei known to receive a projection specifically from PVM (Berk, M.L. and J.A. Finkelstein, J. Comp. Neurol., 220: 127, 1983).

These data indicate some basic similarities in the central connections of nTS at brainstem levels in birds and mammals. However, in the pigeon the nuclear origin of hypothalamic projections to nTS is not the same as the hypothalamic region receiving projections from nTS. In addition, it appears that the organization of visceral connections with more rostral forebrain levels may differ in birds and mammals.

382.12 THALAMIC PROJECTIONS TO THE NONCORTICAL TELENCEPHALON IN A REPTILE. M.B. Pritz and M.E. Stritzel*. Div. Neurol. Surg. Univ. of California Irvine Med. Ctr., Orange, CA 93668

Orthograde degeneration experiments in Caiman crocodilus have identified specific portions of the dorsal ventricular ridge (dorsolateral area) that receive auditory, visual, and spinal, somatosensory input. The present study examined total thalamic projections to noncortical areas of the telencephalon and then focused on thalamic connections with the central portions of the dorsolateral area where thalamic afferents are less well characterized.

Horseradish peroxidase (HRP) injections were placed in noncortical telencephalic areas of 8 juvenile Caiman crocodilus under cold narcosis. After survival periods of 2 to 7 days at water temperatures of 23 to 30°C, animals were given a lethal overdose of sodium pentobarbital and processed for HRP histochemistry with tetramethylbenzidine as the chromogen.

Following large injections of HRP that involved nearly the entire dorsolateral and ventrolateral area, retrogradely labelled neurons were found ipsilaterally in the following thalamic sites: nucleus dorsolateralis anterior, nucleus dorsomedialis anterior, nucleus rotundus, nucleus reuniens, the medialis complex, nucleus diagonalis, and nucleus posterocentralis. Smaller injections restricted to a central portion of the dorsolateral area resulted in intensely labelled cells primarily in the medialis complex and nucleus posterocentralis. Marked filling of neurons in these nuclei enabled visualization of soma shape and pattern of primary dendritic branches.

The number of retrogradely labelled and unlabelled neurons seen after large injections of the dorsolateral and ventrolateral area were further examined in nuclei whose boundaries were easily recognized in neutral red counterstained sections. The cell groups studied were nucleus rotundus and nucleus reuniens pars centralis which receive visual and auditory input respectively from the midbrain, and nucleus dorsolateralis anterior whose afferents are incompletely known. Very few unlabelled cells were present in nucleus rotundus or nucleus reuniens pars centralis. Similarly, few unlabelled cells were seen in nucleus dorsolateralis anterior. However, the unlabelled neurons in nucleus dorsolateralis anterior were somewhat more numerous than those present in nucleus rotundus or nucleus reuniens pars centralis. Although preliminary, these findings of nearly complete retrograde labelling of specific sensory (nucleus rotundus and nucleus reuniens pars centralis) and presumed non-sensory (nucleus dorsolateralis anterior) thalamic nuclei suggest that most of the neurons in these nuclear groups are projection cells and that few exclusively intrinsic neurons are present. If the observations described here are confirmed in other thalamic nuclei in Caiman and in other reptiles, then the development of intrinsic neurons may be an important feature in the evolution of the thalamus.

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382.13 AFFERENT CONNECTIONS OF THE CEREBRAL CORTEX OF THE TURTLE (PSEUDEMYS SCRIPTA ELEGANS). P.H. Desan, Dept. Psych., Univ. of Colorado, Boulder, CO 80309.

The cerebral cortex of the turtle consists of five areas: from lateral to medial, areas L, D2, D1, DM, and M. Their afferent connections were studied with retrograde tracers (HRP, HRP conjugated to lectins, fluorescent beads), and the results suggest homologies between the cortical areas of turtles and mammals.

Injection of retrograde tracers into area D2 labels cells in the anterior nucleus and lateral geniculate nucleus of the thalamus (the latter has been shown to receive optic input: Hall, Foster, Ebner, and Hall, Brain Res. 130:197, 1977), while injections in areas D1, DM, or M label cells in the dorsolateral anterior nucleus of the thalamus. These injections also label cells in thalamic areas which primarily project to the striatum and perhaps resemble the intralaminar nuclei of mammals. Injection of tracer into area L labels cells in the olfactory bulb as well as scattered cells in the dorsolateral anterior nucleus.

Injections in the cortex also label cells in the partly cholinergic nuclei of the basal forebrain (Mufson, Desan, Mesulam, Wainer, and Levey, Brain Res. 323:103, 1984): within the cortex, the nucleus basalis principally projects to area D2, the medial septum to areas DM and M, and the nucleus of the diagonal band to area L.

Finally, area D2 receives projections from the dorsal ventricular ridge, dorsal sector, and area D1 from all of the sectors (these sectors are known to receive projections from thalamic sensory nuclei: Balaban and Ulinski, J. Comp. Neurol. 201:95, 1981). Areas DM and M receive input from the medialmost strip of area L and from area D1.

Thus, both areas D2 and L receive primary sensory input from the visual thalamus and the olfactory bulb, respectively: Area D2 may be homologous to mammalian primary visual neocortex and area L to mammalian primary olfactory cortex. Area D1 receives its main input from area D2 and other thalamorecipient sensory areas: it is perhaps homologous to mammalian polysensory association cortex. Areas DM and M receive convergent olfactory information via a medial part of area L and thalamic sensory information via area D1: areas DM and M are thus similar to the mammalian hippocampus. Indeed, the projections from areas L and D1 resemble the mammalian lateral and medial perforant paths (Desan, Soc. Neurosci. Abstr., 1981), suggesting that these areas correspond to the lateral and medial entorhinal areas of mammals. The differential projections of the cholinergic basal forebrain nuclei are consistent with this division of the turtle cerebral cortex into "paleocortical," "neocortical," and "archicortical" zones.

## COMPARATIVE NEUROANATOMY II

383.1 AN IMMUNOHISTOCHEMICAL STUDY OF THE TELENCEPHALON OF THE AFRICAN LUNGFISH. R.G. Northcutt and A. Reiner. Division of Biological Sciences and Department of Anatomy and Cell Biology, The University of Michigan, Ann Arbor, MI 48109.

Lungfish are among the last living members of the group of bony fish that gave rise to terrestrial vertebrates. Studies of lungfish can thus shed light on the evolution of the brain during the vertebrate transition to land. In this immunohistochemical study, antisera against the following neuroactive substances were used to study the telencephalon of the African lungfish, Protopterus: 1) serotonin (5HT, Immunonuclear Corp.); 2) tyrosine hydroxylase (TH), a marker for catecholamines (T. Joh); 3) substance P (SP, Accurate Chemical); 4) leucine-enkephalin (LENK, Immunonuclear Corp.); 5) avian pancreatic polypeptide (APP, J. R. Kimmel); and 6) LANT6 (R. E. Carraway).

In lungfish, the ventrolateral telencephalic wall, like that of amniotes, possesses 1) a "striatal" region rich in SP+ and LENK+ neurons and fibers and appears to give rise to a descending SP+ pathway that terminates on tegmental TH+ neurons that appear to have a reciprocal projection to the "striatum", and 2) a caudally situated "pallidal" field of large LANT6+ neurons. The ventromedial wall of the telencephalon contains a more ventral region that is rich in SP+, LENK+ and LANT6+ neurons and in TH+, 5HT+ and APP+ fibers, and a more dorsal region rich in SP+, LENK+, LANT6+, 5HT+, TH+ and APP+ fibers. In these characteristics, these regions are comparable to the nucleus accumbens septi and the septal nuclei, respectively, in amniotes.

The dorsomedial wall of the telencephalon (termed the medial pallium) comprises three immunohistochemically distinct cell groups: 1) a dorsal cell group distinctively rich in SP+ and LENK+ fibers; 2) an intermediate cell group distinctively rich in APP+, 5HT+ and TH+ fibers and APP+ neurons; and 3) a ventral cell group with a moderate number of 5HT+, TH+, SP+, LENK+ and APP+ fibers and APP+ neurons. These results do not provide unequivocal data as to correspondences between cell groups of the medial pallium in amniotes and lungfish. Finally, the rostral dorsolateral wall of the telencephalon comprises the caudal pole of the olfactory bulb, as reflected in the large number of TH+ neurons. In more caudal portions of the dorsolateral wall, most of which corresponds to olfactory cortex, no immunohistochemically distinct region that might be comparable to mammalian isocortex or sauropsid DVR was evident.

In summary, these results indicate that while the evolution of the ventral telencephalon from bony fish to land vertebrates was characterized by a striking degree of conservatism, a similar degree of conservatism is not evident for the dorsal telencephalon.

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383.2 CEREBELLAR AFFERENTS IN THE GREEN SUNFISH, LEPOMIS CYANELLUS. M. F. Wullimann* and R. G. Northcutt (SPON: M. S. Northcutt). Division of Biological Sciences, University of Michigan, Ann Arbor, MI 48109.

The pretectum of perciform teleosts is one of the most complex neural regions to develop in the course of vertebrate brain evolution. Many of the pretectal nuclei receive retinal and/or tectal inputs, but few details are known regarding the efferent projections of these cell groups. Several experimental studies on bony fish species with more primitive pretecta indicate that a number of these nuclei project to the cerebellum. Horseradish peroxidase (Sigma VI) was injected unilaterally into the corpus (8 cases) of green sunfish in order to determine pretectal and additional inputs to the cerebellum. Following survival times of 2-4 days at 22-24°C, the animals were reanesthetized and perfused transcardially with cold phosphate buffer (pH 7.4) followed by 4% glutaraldehyde in phosphate buffer. Brains were removed, embedded in 25% gelatin, and cut at 35µm. Sections were reacted with o-dianisidine, tetramethyl benzidine or Hanker-Yates reagents.

Unilateral cerebellar injections retrogradely labeled cells in the following diencephalic and pretectal nuclei: ipsilaterally in the dorsal (basal optic nucleus of Braford and Northcutt, '83) and ventral (accessory optic nucleus of Braford and Northcutt, '83) accessory optic nuclei, central and periventricular pretectal nuclei, and paracommissural nucleus of Ito et al. ('82). At midbrain levels, retrogradely labeled cells were seen ipsilaterally in nucleus isthmi, lateral nucleus of the valvula, perilemniscal nucleus, and bilaterally in the dorsal tegmental nucleus (lateral tegmental nucleus of Braford and Northcutt, '83). At more caudal levels, retrogradely labeled cells were seen contralaterally in the inferior olivary nucleus and the ventral and intermediate gray columns of the spinal cord. Cells of the lateral reticular nucleus, inferior raphe, locus coeruleus, and nucleus of the commissure of Wallenberg were labeled bilaterally.

Our results suggest that the cerebellar corpus of sunfish receives substantial telencephalic and visual inputs. The paracommissural and dorsal tegmental nuclei are known to receive telencephalic inputs, and both nuclei project to the corpus in sunfish, as in other ray-finned fishes. The central and periventricular pretectal nuclei, as well as the dorsal and ventral accessory optic nuclei, receive direct retinal input (Butler and Northcutt, '81) and also project to the corpus of the cerebellum. There are extensive tectal projections to many of these nuclei (Northcutt, '82), as well as cerebellar projections to the optic tectum, which suggests that the pretectum, tectum, and cerebellum are complexly involved in visually mediated behaviors.

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383.3 BRAINSTEM NEURONS PROJECTING TO THE SPINAL CORD IN GOLDFISH: AN HRP STUDY. H.E. Sloan and L.S. Demski. School of Biological Sciences, University of Kentucky, Lexington, KY 40506.

Gelfoam pledgets containing HRP (Sigma VI) were placed at either rostral (3-8), middle (14-16) or caudal (24-26) vertebral levels of transected or hemisected SC in adult goldfish (10-14 cm standard length). Following survival times of 3, 4, 7 or 9 days at 26±2°C animals were perfused and brains were sectioned in either transverse, horizontal or sagittal planes. Tissue was processed using Hanker-Yates or TMB protocols and examined with light microscopy. Diencephalic cells which project to SC occur in the preoptic area (see Sloan and Demski, Soc. Neurosci. Abstr. p. 854, 1984), zona limitans-dorsal hypothalamic area as well as the periventricular thalamic and the posterior tuberal nuclei at the level of the posterior commissure. Cells of the posterior tuberalis project bilaterally while the other filled cells have ipsilateral connections. Labeled mesencephalic cells are in the nucleus of the medial longitudinal fasciculus, (nMLF), a nucleus ventrolateral to nMLF in the rostral tegmentum, and an isthmal tegmental nucleus ventral to the nucleus lateralis valvulae. The majority of labeled cells in the rostral tegmental nucleus are contralateral to the implant while other labeled nuclei have bilateral projections. Rhombencephalic cells distributing fibers to SC occur in reticular nuclei (superior, medial and inferior), inferior raphe, nucleus of the descending trigeminal tract, octaval nuclei (magnocellular, descending, and tangential) and the facial lobe. Labeled cells of the facial lobe project ipsilaterally while cells of other nuclei have bilateral connections with a larger ipsilateral component. Labeled cells were absent from the vagal lobes, cerebellum, optic tectum and telencephalon. Supported by NIH grant NS 19431-02.

383.4 THE PRIMARY PROJECTIONS OF THE TRIGEMINAL NERVE IN THE GOLDFISH, CARASSIUS AURATUS. R. L. Puzdrowski, Div. Biol. Sci's., Univ. of Mich., Ann Arbor, MI 48109.

The central projections of individual rami of the sensory component of the trigeminal nerve were traced with HRP. Adult goldfish (8-14cm total length) were anesthetized with MS222, and individual rami were exposed and transected. A gelfoam pledget soaked with 40% HRP (Sigma VI) was placed on the proximal stump of the transected branch. After a survival time of 7-11 days at 22-26°C, the animals were reanesthetized and transcardially perfused with phosphate buffer (pH 7.4) followed by a 2% glutaraldehyde solution. The brains were sectioned transversally at 40µm and processed according to the Mesulam ('78) TMB protocol or by the Hanker-Yates ('77) protocol.

The sensory rami of the trigeminal nerve of goldfish are distributed over the head through three nerve trunks, the supraorbital, infraorbital and hyomandibular.

The fibers of the trigeminal sensory nerve enter the medulla as a single root accompanied by the trigeminal motor nerve. The sensory component caps the ascending secondary gustatory tract and courses caudally as the descending trigeminal tract (DTV). As described in carp (Luiten, '75), the DTV was found to project ipsilaterally to five termination sites. Four of the sites lie dorsomedially to the branchiomeric motor nuclei. The fifth site, the medial funicular nucleus (NMF), receives the bulk of the fibers of the DTV. Each trigeminal ramus was found to project to all five terminal sites. In general the rami of the infraorbital and hyomandibular trunks terminate ventrally to the ramus of the supraorbital trunk in the terminal sites dorsomedial to the branchiomeric motor column. This projection pattern is reversed in the NMF where the supraorbital trunk projects to the ventral portion of the nucleus, the infraorbital trunk to the dorsal portion of the nucleus, and the hyomandibular trunk to the dorsolateral portion of the nucleus. In addition to the above projections, filling of the infraorbital or hyomandibular trunks results in a minor projection from the ventral bundles of the descending facial sensory root to the terminal site at the level of the seventh motor nuclei.

Labeling of trigeminal mesencephalic cells occurred only with filling of the supraorbital or infraorbital trunks. The cells of the mesencephalic nucleus are distributed mediolaterally along the floor of the optic ventricle in the region of the posterior commissure. Following filling of the infraorbital trunk, labeled cells were observed throughout the nucleus. In contrast, filling of the supraorbital trunk results in labeling of only medially situated cells.

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383.5 PRIMARY TRIGEMINAL PROJECTIONS IN THE PACIFIC HAGFISH M.C. Ronan Dept. of Biology, Wesleyan University, Middletown, CT 06457

The central projections of primary afferents comprising major nerves of the trigeminal complex in the Pacific hagfish, Eptatretus stouti, were examined by transganglionic transport of horseradish peroxidase (HRP). In adult hagfish, 28-57 cm long, one or more branches of the opthalmic (17 cases), externus (12 cases), or dentalis (4 cases) nerves were uni- or bilaterally transected distal to their ganglia. Gelfoam pledgets, saturated with concentrated HRP (Sigma VI), were placed on the proximal nerve stumps. In three other animals, the facial or vagus nerves were similarly labeled. Animals survived 14-22 days at 10-14°C. After transcardial perfusion with 4% glutaraldehyde, the fixed brains were sectioned transversely at 40 µ and processed according to a modification of the Hanker-Yates procedure.

The nerves of the trigeminal complex join the brain stem in the prominent rostrolateral horns of the Y-shaped medulla. HRP-filled trigeminal afferents continue caudally in the large descending trigeminal tract of the dorsolateral medulla. Within the trigeminal tract, afferents associated with the major trigeminal nerves segregate into separate fascicles, each surrounded by a thick band of densely packed cells. Opthalmic afferents are situated in the dorsal and dorsomedial tract; externus afferents are found immediately lateral to the opthalmic field. Dentalis afferents are located ventrolaterally. In a single clearly labeled case for each, facial nerve fibers were seen to terminate in the dentalis field while primary vagal afferents coursed along the ventral and lateral borders of the same field. Heavily-labeled fibers in all trigeminal fascicles were traced as far as the rostral spinal cord.

The opthalmic, externus, and, to a lesser extent, the dentalis fields include both a medial, coarse-fiber fascicle and a separate lateral, fine-fiber fascicle, perhaps indicating the presence of two modalities in each nerve. Afferents in the most caudal branch of the opthalmic nerve are confined to the dorsal portions of both the medial and lateral opthalmic fields. Tract fascicles preserve their integrity throughout the extent of the trigeminal tract, but are further partitioned at more caudal tract levels by thin bands of cells. HRP injections of the midbrain reveal the neurons in the tract cellular bands as one source of ascending 2° trigeminal projections. These cells lying among the afferent fibers are likely the nucleus of the descending trigeminal tract.

Anatomical evidence suggests that the somatosensory system of the head, unlike the visual and octavolateralis systems, is well developed in hagfishes. The trigeminal tract occupies 20-25% of the total medullary volume, appears to be somatotopically organized, and may contain multiple modalities. (Supported by a Grass Fellowship at Friday Harbor Laboratories.)

383.6 LAMINATED AREA POSTREMA OF GOLDFISH: ULTRASTRUCTURE, IMMUNOREACTIVITY AND AFFERENTATION. Y. Morita and T.E. Finger. Dept. of Anatomy, Univ. Colorado Med. Sch., Denver, CO 80262.

The area postrema is the most caudal of the circumventricular organs. This specialized nucleus in the dorsocaudal medulla is well vascularized and is characterized ultrastructurally by fenestrated sinus capillaries and abundant connective tissue elements in a broad perivascular space. The proposed functions of the area postrema involve homeostatic reflex responses which are triggered by blood-borne chemicals, including functions such as emesis, osmoregulation, and cardiovascular control.

In goldfish, the area postrema is a fused midline structure extending along the dorsal surface of the caudal medulla. The area postrema is adjacent to the underlying commissural nucleus of Cajal which serves as the primary general visceral sensory nucleus. Although many of the ultrastructural features of the area postrema in goldfish are similar to those of other vertebrate species, the area postrema in goldfish is of interest in terms of being organized as a laminar system: meninx, vasculature, pallisade region, neuronal somata, and ventral neuropil. The pallisade region contains neuronal processes extending upward among more numerous astrocytic processes. These glial processes exhibit invaginations and coated vesicles typical of pinocytosis. In addition, vescicular and tubular membranous structures frequently occur within the glial processes.

Most neurons of the area postrema exhibit tyrosine-hydroxylase-like immunoreactivity. Each neuron has two types of dendritic specializations. A short foot process extends dorsally to reach the external basal lamina of the capillaries. No synaptic specializations occur on these foot processes, so the probable function of these processes is detection of blood-borne chemicals which leak out from the fenestrated capillaries. The other class of dendrites arising from the neurons of the area postrema are long, thick, basal dendrites which extend ventrally into the commissural nucleus of Cajal. These ventral dendrites receive numerous synaptic contacts, both within the ventral neuropil of the area postrema and within the commissural nucleus. At least two morphological types of terminals can be identified contacting the ventral dendrites. Some primary afferent fibers of the vagus nerve terminate within the area postrema as well as in the commissural nucleus. Thus neurons of the area postrema can act not only as direct chemoreceptive interoceptors, but may also receive input from other elements of the peripheral and central nervous system.

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383.7 ORGANIZATION OF THE PRIMARY GENERAL VISCERAL SENSORY NUCLEUS IN THE GOLDFISH: ORGAN REPRESENTATION AND IMMUNOCYTOCHEMISTRY. T.E. Finger and Y. Morita. Dept. of Anatomy, Univ. of Colo. Med. Sch., Denver, CO 80262.

In many species of fish, such as the goldfish Carassius auratus, the general visceral sensory nucleus, or commissural nucleus of Cajal, is clearly distinct from the more rostrally situated special visceral (gustatory) centers. This allows for ready separation of general visceral from gustatory systems within the brainstem. The commissural nucleus receives direct input from primary sensory fibers of the vagus nerve roots which innervate the viscera (Morita et al., J.Comp.Neurol. '80). In the present study, HRP was used to label various peripheral branches of the vagus nerve or their endorgans. The commissural nucleus also was examined by immunocytochemical means in order to determine cytological boundaries.

The commissural nucleus is broadly divisible into two principal zones, medial and lateral. The medial zone lies caudomedial and ventral to the lateral zone, and is characterized by high levels of immunoreactivity to supstance P, tyrosine hydroxylase, serotonin and FMRF-amide antisera. Some somata immunoreactive for tyrosine hydroxylase also occur within the medial zone. Some of the substance P-like immunoreactivity in the caudal portion of the medial zone is diminished following transection of the vagus nerve. The substance P-immunoreactive terminals in this area form asymmetric synapses onto dendritic elements of the medial zone.

HRP labeling studies demonstrate that the medial subnucleus contains vagus nerve terminals of fibers which innervate the subdiaphragmatic (actually, sub-transverse septum) viscera. Sensory fibers which innervate supradiaphragmatic structures such as the heart, posterior pharynx, and esophagus terminate in the lateral subnucleus of the commissural nucleus. In addition, a small number of primary afferent vagal fibers project bilaterally to the area postrema and dorsal motor nucleus of the vagus. The visceral motor neurons innervating the supradiaphragmatic vagus tend to be segregated from those innervating subdiaphragmatic branches of the nerve. There are approximately 30 cardiac preganglionic parasympathetic neurons in a 15-20 cm goldfish and are located in a diffuse column situated just medial to the main body of the dorsal motor nucleus of the vagus.

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383.8 MORPHOLOGY OF THE MACULA NEGLECTA IN THE BOWFIN, AMIA CALVA. W.M. Saidel and C.A. McCormick. Dept. of Anatomy, Georgetown Univ., Washington D.C., 20007.

The morphology of the macula neglecta (mn) of the bowfin, Amia calva was studied using light and scanning electron microscopy, and its central connections were determined using horseradish peroxidase (HRP) tract tracing methods. The mn consists of two asymmetric patches of sensory epithelia located at the base of the common crus immediately caudal to the utriculo-saccular duct in the posterior semicircular canal. The medial sensory patch forms a ridge across the canal and the lateral patch protrudes from a bulge in the canal's lateral wall. A gelatinous cupula overlies the sensory epithelia.

The ciliary bundles of most hair cells (hc) in the mn are characterized by long kinocilia and shorter stereocilia similar to those seen in semicircular canal cristae, and unlike those seen in otolithic endorgans. A kinocilium is located at the caudal pole of the ciliary bundle of hc's in the medial patch and at the rostral pole of hc's in the lateral patch. The surface area of the lateral patch is 60% of the medial patch (n=4), although the absolute areas varied in different specimens. Hc number was estimated in each patch by counting the number of hc bases in sonicated tissue. Hc density averaged 788±100/(100μm)$^2$ (n=18) in the medial patch and 880±92/(100μm)$^2$ (n=9) in the lateral patch, leading to a total number of about 8100 in an adult mn. 132±10 (n=4) nerve fibers innervate the mn for a convergence ratio of about 61 hc's/nerve fiber.

The distal portion of the mn nerve bifurcates into 2 branches, each branch innervating one sensory patch. The two branches merge proximally and join the posterior ramus of the eighth nerve. The proximal portion of the mn nerve was impaled with an HRP-coated minuten insect pin in 5 additional anesthetized Amia. After 6-10 days survival (28°C), the brains were processed as in a previous study (McCormick,'81). The mn nerve projects to the ventral divisions of the four octavus nuclei present in Amia and to the medial portion of the eminentia granularis. The pattern of distribution is similar to that of the posterior semicircular canal nerve. However, the medullary projection from the mn is very sparse, the majority of mn fibers terminating in the eminentia.

The macula neglecta is found in at least some species of all vertebrate classes, but its function has been little studied. Its function is unknown among bony fish, but its morphological features in Amia suggest a vestibular function.

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383.9 ASCENDING CONNECTIONS OF THE TORUS SEMICIRCULARIS IN THE TADPOLE, RANA CATESBEIANA. K. Rubinson and C. Brown*. Dept. of Physiology & Biophysics, NYU Medical Center, New York, NY 10016.

Previous studies have indicated the contemporaneous existence of distinct acoustic and lateral line nuclei in the premetamorphic bullfrog (Jacoby, J. and Rubinson, K., J. Comp. Neurol., 216:152-161, 1983). Prior to the involution of the lateral line system, we have demonstrated descending efferent projections from the torus semicircularis (Jacoby J. and Rubinson, K. Brain Res., 292:378-381,1984). These toral projections appear to involve both acoustic and lateral line areas of the brainstem. To date, no demonstration of a toral-thalamic projection has been reported for the tadpole although such is known to exist in the adult bullfrog (Wilczynski, W., J. Comp. Neurol., 198:421-433, 1981).

We have injected horseradish peroxidase (HRP) into the torus semicircularis of bullfrog tadpoles, at Taylor and Kollros stages II-XI. In these various animals, we have traced ascending projections from the subnuclei of the torus to the ipsilateral thalamus with heavy terminal labelling in the central thalamic nucleus and the lower portion of the lateral thalamic nucleus. A small region of terminal labelling was found in the posterior entopeduncular region. In addition, filled cells, indicating neurons which project to the torus, were found in the posterior thalamic nucleus with dendrites extending laterally into and through the lateral nucleus. A few retrogradely filled cells were found on the medial edge of the central nucleus. No filled cells were found in the lateral nucleus.

This pattern is similar but not identical to that described for the auditory torus of adult bullfrogs. The additional regions of termination and filled cells may be attributable to the still functional lateral line system.

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383.10 ORGANIZATION OF THE RETICULO-SPINAL PROJECTION FROM THE ISTHMIC REGION IN THE FROG (Rana esculenta). B.G.Grover* and U.Grüsser-Cornehls* (SPON: European Neuroscience Association). Inst. of Physiol., Freie Universität Berlin, West Berlin, FRG.

It is difficult to compare the cytoarchetecture of the reticular formation in anurans to that in sauropsids and mammals. Most reticular cells in the former are found in or at the periphery of the central grey. Further, the distortion of the mes- and metencephalon resulting from the great development of the optic tectum make topographic comparison difficult. In the present study the distribution and dendritic morphology of reticular cells labelled after spinal applications of HRP or WGA-HRP were investigated, with particular attention to the mes- and metencephalon. Applications of tracer were made either by injection into the spinal grey via micropipettes or by lesioning the white matter with a tracer coated razor blade fragment. Additional cases were available in which injections had been made into the tectum and basal forebrain. Survival times of eight to 14 days were allowed. Alternate serial sections were processed on the slide with DAB and BDHC or TMB.

Axons of cells in the laminar nucleus of the torus semicircularis (TSl) and the anterodorsal (AD) and posteroventral (PV) nuclei of Potter descend ipsilaterally to at least lumbar levels and appear to terminate at all levels, principally ipsilaterally. Labelled cells are found in the rostral 3/4 of TSl but not in its medial or lateral parts. The rostral pole of AD is found at the level of midline fusion of the tori semicirulare. At this level cell labelling in TSl and AD is contiguous. Cell size and primary dedritic structure in TSl and AD are very similar. Further, both areas receive fibers from the tectum and forebrain, although fibers to TSl are sparser. These findings suggest that TSl and AD may represent extrusions of the same population of cells. The TSl-AD complex resembles, in its connections and topological relations, the intercollicular nucleus of sauropsids and the cuneiform area of mammals.

Labelling in AD is found ipsilateral to spinal injections. In the rostral 2/3 of PV labelling is primarily ipsilateral and found in the same locations on both sides. Caudal to the nucleus isthmi, ipsilateral labelling in PV tends to be found medially and contralateral labelling laterally. Labelled cells in the white matter of the isthmus were found contralateral to spinal injections. Although various nuclei of the upper pons may be represented in this area, more information is necessary to determine what parallels to amniote organization might exist.

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383.11 STUDIES ON THE TERMINAL NERVE OF ELASMOBRANCH FISHES. L.S.Demski, R.D.Fields and T.H.Bullock. School of Biological Sciences, Univ. of Kentucky, Lexington, KY 40506 and Neurobiology Unit, Scripps Institution of Oceanography, La Jolla, CA 92093

Based on light microscopic analysis in stingrays (Urolophus halleri) and thornbacks (Platyrhinoidis triseriata), the terminal nerve (TN) consists of three ganglia distributed along a nerve that is separate from the olfactory tract (OT). A distal ganglion sits on a vascular sinus on the rostrodorsal surface of the olfactory bulb (OB). Its branches follow the sinus along the margin between the OB and the olfactory epithelium. Proximal ganglia are located between the OT and the surface of the forebrain. The more distal one is spindle-shaped and grades into the other. The most proximal ganglion or "white body" (WB) is round and has a lobular appearance with clusters of large cells separated by fibers of the TN. It sits on a vascular sinus on the forebrain surface and has branches that either extend along nearby vessels or enter the telencephalon directly. At the EM level the TN of the rays and the dogfish (Squalus acanthias) is characterized by many non-myelinated axons with diameters of .25-1.2µm. Many of the fibers contain clusters of dense core vesicles (DCV) with diameters to about .2µm. Neurons in the WB of dogfish have a multilobular nucleus with cytoplasm full of DCV. Synapses with clear vesicles occur in the ganglia and adjacent nerve. A few larger processes (up to 2.7µm) were observed in stingrays. They also contained DCV and were sometimes surrounded by multiple cytoplasmic membranes. In stingrays nerve processes (.8-2.5µm) appear to leave the TN and enter perivascular spaces. These endings also contain abundant DCV and resemble the large processes described above. Transfer of material from the nerve fibers to the vessels is suggested. Unit and multiunit responses were recorded from the TN-complex in stingrays using glass and metal microelectrodes. Fish were anesthetized in MS 222 and then immobilized with d-tubocurarine. Most units fired at a rather slow steady rate varying from 2 to about 8/s. In a few cases however, bursts of up to 15 to 30 or more/s occurred for periods up to 3-4s. Attempts to influence the activity of these units with light flashes, sounds in air, rubbing of skin, infusions of chemical solutions in the nares and water movements over the body were unsuccessful. Supported by NIH and NSF grants.

383.12 SPINAL PROJECTING NUCLEI IN THE BRAINSTEM OF AN ELASMOBRANCH, THE THORNBACK GUITARFISH, PLATYRHINOIDIS TRISERIATA. W.L.R. Cruce and R.G. Northcutt. Neurobiology Program, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272 and Division of Biological Sciences, University of Michigan, Ann Arbor, MI 48109.

It has recently been hypothesized (Cruce and Newman,'84, Amer.Zool.24: 733-753) that the reticulospinal nuclei of non-mammals are homologous to those of mammals. This hypothesis emerged from work in reptiles (e.g., Newman, et al.,'83, JCN 215: 17-32) and should be tested in representatives of other vertebrate classes. We have begun this by looking at an elasmobranch where we have found enough distinctly different spinal projecting nuclei that we believe we can recognize mammalian homologies. We have indicated these homologies by adopting, particularly for reticular nuclei, the mammalian names (using those of Newman,'85, J.Hirnfsch. 26(2)). These constitute a working hypothesis of homologies to be tested in the future, e.g. by investigating transmitter immunocytochemistry, afferent connections or ascending efferent connections.

Animals received unilateral placements of horseradish peroxidase (dried on a pin) into various levels of the spinal cord. After survival periods of 5-34 days, the animals were perfused, the brains removed and sectioned, and alternate sections were reacted with the tetramethyl benzidene or Hanker-Yates procedures. Sections were analyzed with the aid of a computer-microscope drawing system.

The following nuclei were identified and found to project to the spinal cord bilaterally (b), ipsilaterally (i) or contralaterally (c): reticularis dorsalis (b), reticularis ventralis (b), descendens nervi trigemini (b), ambiguus (c), solitarius (i), reticularis gigantocellularis (b), reticularis magnocellularis (i), raphe magnus, reticularis paragigantocellularis (i), octavus descendens (b), reticularis pontis caudalis pars a (i), reticularis pontis caudalis pars b (b), octavus magnocellularis (i), nucleus "B", reticularis pontis oralis pars medialis (i), reticularis pontis oralis pars lateralis (c), coeruleus-subcoeruleus (b), parabrachialis (Kolliker-Fuse) (c), mesencephalicus lateralis (c), pedunculo-pontine pars compacta (i), pedunculo-pontine pars dissipata (c), ruber (c), interstitialis fasciculi longitudinalis medialis (i), accessory oculomotor (Edinger-Westphal) (b), periventricularis pretectalis pars dorsalis (c), posterior tuberculum (i), ventromedialis thalami (i). Previously the majority of these nuclei have been grouped within the superior, middle, and inferior reticular nuclei. This is the first report in any fish of spinal projecting brainstem nuclei with a level of complexity comparable to that of mammals. Supported in part by grants from the Stark County United Way (WLRC) and NIH grants NS11006 and EY02485 (RGN).

383.13 PECULIAR INTRACRANIAL CIRCULATION IN AN ELASMOBRANCH: THE RETE MIRABILE CRANICA IN THE GENUS MOBULA. J. Schweitzer and G. Notarbartolo-di-Sciara*. Neurobiology Unit, Scripps Institution of Oceanography, Univ. of Calif., San Diego, La Jolla, CA 92093

For two centuries cranial blood supply has been studied in diverse species of batoids and sharks, yet the unusual cephalic circulation in the highly specialized devil rays (Mobulidae; genera Manta and Mobula) has received little attention. We describe the morphology of the mobulid rete mirabile cranica (RMC) using histological, scanning electron microscopic, and plastic corrosion cast techniques. It is a massive arterial network grossly divisible into a "caudal RMC" supplying blood to the brain and an expanded more complex "precerebral RMC" nested within the large cranial cavity rostral to the brain. The precerebral rete in a 45 kg M. thurstoni with a 100 g brain weighs 40g (compare to a 30 g brain in a nurse shark (Ginglymostoma cirratum, no rete) of the same weight).

Although important interspecific differences are apparent among the retia of the 4 Mobula species studied, all share a common plan. The RMC originates from the posterior portion of the profundae cerebri arteries which lie ventral to the brain and form the sides of a vascular triangle, the base of which is anterior and formed by the joining of the paired internal carotids; the vertex is posterior and median, corresponding to the anterior extreme of the spinalis impar artery. Blood enters the spinalis impar from the paired dorsal aortae through small communicating vessels and from there diverges to flow both cranially and caudally. Cranial blood is supplied exclusively from the spinalis impar and paired internal carotids and enters the brain through vessels of the caudal rete which branch posteriorly from both profundae cerebri. Vessels branching more anteriorly course rostrally to form the precerebral RMC, a massive network of convoluted arteries (each ca. 1mm diameter with thick muscular walls) encased in a gelatinous matrix anterior to the telecephalon. Within the gelatinous matrix the precerebral RMC is a paired structure divided along the midline, across which the 2 sides do not communicate except by means of a single rostral transverse artery. The precerebral rete takes the shape of the cranial cavity and completely envelops the olfactory nerves to the level of the olfactory bulbs at its anterolateral extreme. Some of the large vessels taper and branch to ca. 160 um, forming a secondary arterial system. Many of the these smaller arteries course parallel to the primaries and give rise to a mesh of finer (ca. 30 um) tertiary vessels which ensheath the largest arteries. Both secondary and tertiary vessels are embedded in the adventitia of the primaries, with which they communicate by numerous arterioarterial anastomoses.

The RMC clearly is not a counter-current device, but its tremendous complexity and fine structure suggest some specialized function(s). The secondary arterial system may be involved in plasma skimming and the production of cerebrospinal fluid; significantly, in mobulids the lateral ventricles, and probably the choroid plexuses, are greatly reduced.

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383.14 NEW VIEWS ON THE NEUROPILE OF THE EYESTALK OF THE PROCAMBARUS CLARKI. J.P. Machado-Salas, B. Pérez*and J.Espinosa* Lab. Neuromorfología.Neurociencias.UIICSE.ENEP Iztacala.UNAM.Tlalnepantla 54030.Edo. de México.MEXICO.

While studying some structural aspects of plasticity shown by the neurosecretory system of the eyestalk of the P.clarki,we realized that the structural and ultrastructural features of this organ was far from been fully studied.Actually some of the available data is null or scarce and in some respects is oudated.For this reason, we have used silver stains and some ancillary neurohistological techniques to study the normal eyestalk of this particular crayfish, as an adequate prelude for further ultrastructural studies.

The eyestalks of mature female crayfish were sectioned and immediately fixed in buffer-formaldehyde;afterwards, some eyestalks were immersed in the Golgi solution (osmic-dichromate) and the remaining tissue was processed with PAS, luxol-fast-blue and Nissl's techniques.In all cases, we made transverse and longitudinal sections.

Our results have shown: a) a rather complex vascular pattern, which is highly sophisticated at the level of the medulla externa, b) here, we saw two rows of cell nests surrounded by vascular meshes, c) high cellularity of the external medulla contrasts with the almost acellular panoram shown by the twoother medullas, d) the peripheral cellular systems, that surround the core of the eyestalk and includes the X-organ, showed three types of neurons:small, medium and large, e) the latter are few and some of them are strongly positive for PAS stain. f) the nerve fibers that originate at the inferior portion of the external medulla, develop a bipenate pattern, which repeats in every section as 3 long and 4 to 6 short bipenated columns, g)the sinus gland shows the typical loose vascular pattern and the presence of neurosecretory granules, h) finally, the 140 microns-thick Golgi sections, have provided further data on: the penetration of the retinal fibers, the vasculo-cellular relationships, a rich variety of nerve cells which processes cover a full range of anatomical varieties and a better understanding of the tridimensional features of these neuropiles.

These data shall be discussed in terms of current physiological knowledge of this structure.

384.1 EPILEPTOGENIC EFFECTS OF CARBACHOL MICROINJECTED IN THE BRAINSTEM. Z. Elazar. Dept. of Physiology and Pharmacology, Sackler Medical School, Tel Aviv University, Israel, 69978.

Experiments were performed in chronic rats to determine the sensitivity of the brainstem reticular formation to the convulsant effect of carbachol. Carbachol was microinjected in hippocampus, mesencephalic reticular formation (MRF) and pontine reticular formation (PRF). EEG activity from hippocampus, MRF, PRF, motor cortex and the site of microinjection was monitored. Cervical EMG was also monitored.

In hippocampus, amounts of carbachol at threshold for local electrical seizures (0.5-1 μg in 1 μg saline) induced immobility or behavioral excitation. Higher amounts of carbachol induced clonic convulsions. Hippocampal seizures mostly spread to reticular formation.

In MRF, amounts of carbachol 200-500°/o higher than in hippocampus were necessary to induce local electrical seizures, which were similar graphically to hippocampal seizures. MRF seizures spread to PRF, hippocampus and motor cortex. These seizures could be accompanied by the following motor phenomena: immobility, behavioral excitation, facial clonies, forelimb clonies, whole body clonic jerks and generalized clonic convulsions.

Amounts of carbachol similar with those inducing Grand Mal convulsions when injected in MRF, induced when injected in PRF signs of REM sleep: local EEG desynchronization, cortical desynchronization, sustained theta waves in hippocampus and muscle atonia. The threshold for local electrical seizures and convulsions was in PRF 200-400°/o higher than in MRF.

Intracerebral injections of 6-OHDA lowered the threshold for PRF local seizures and convulsions.

We conclude as follows: 1. local organized electrical seizures can be induced in the brainstem reticular formation by chemical stimulation. This is in contrary to results published so far according to which electrical stimulation of the reticular formation induces convulsions but not local EEG seizures. 2. PRF has a higher threshold for epilepsy than MRF. 3. Locus coeruleus noradrenergic mechanisms control the sensitivity to epilepsy of the PRF. 4. REM sleep or epilepsy can be induced from the pons depending on the intensity of chemical stimulation.

384.2 SYNCHRONOUS PAROXYSMAL DISCHARGES IN NEOCORTICAL SLICES FROM CHEMICALLY KINDLED RATS. E. Barkaie*, A. Friedman*, R. Lobel-Yaakov* and M.J. Gutnick*, (Spon: R. Foster) Unit of Physiology, Faculty of Health Sciences, Ben Gurion University of the Negev, Beersheva, Israel.

Kindling refers to an experimental model of epilepsy in which repetitive daily delivery of low-intensity electrical or chemical stimuli gradually induces a permanent change in the epileptogenic sensitivity of forebrain structures, such that initially subconvulsive stimuli become capable of evoking paroxysmal activity. The purpose of the present study was to determine whether the kindled state in neocortex is correlated with consistent changes in the activities of individual neurons. Intracellular recordings were made in neocortical slices from rats that had previously been chemically kindled.

Fifteen adult rats were kindled by systemic administration of an initially subconvulsive dose of pentylenetetrazol (PTZ) (30 mg/kg ip every 48 hrs). After each injection, the animal's convulsant response was classified on a scale of 1-4. The first PTZ injection never caused any obvious convulsive effect; severity of subsequent responses increased progressively and class 4 seizures were observed in all animals by the 7th-20th injection, at which time regular PTZ injections were discontinued. In each animal, a single injection of 30 mg/kg PTZ still evoked a class 4 response after a drug free period of a month or more. An equivalent number of litter mates received saline injections. Intracellular recordings were made in slices of parietal neocortex (400 μm thick) that had been prepared from kindled and control rats and maintained *in vitro* at 36°C in oxygenated Ringer solution (2 mM Ca, 5 mM K). Recordings and data analysis were done blind.

In slices from all kindled rats, but not from control rats, low intensity electrical stimuli to the white matter evoked all-or-none paroxysmal extracellular field potentials which occured in synchrony with intracellularly recorded depolarization shifts and superimposed spike bursts. The characteristics of these kindling-related paroxysmal responses were very similar to those previously reported for epileptogenesis in neocortical slices exposed to convulsant agents.

Intracellular injections of the fluorescent dye lucifer yellow CH were made to test whether the kindled state might be associated with an increased tendency for neocortical neurons to be dye-coupled, and hence, electrotonically coupled. While dye-coupling was present in slices from kindled animals, its incidence was no greater than in control.

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384.3 DURATION OF POST-ICTAL REFRACTORINESS IN THE GENETICALLY EPILEPSY-PRONE RAT (GEPR). C. E. Reigel, Jr.*, J. W. Dailey, J. A. Ferrendelli and P. C. Jobe. Department of Basic Sciences, University of Illinois College of Medicine at Peoria, Peoria, IL 61656, Department of Pharmacology, Louisiana State University Medical Center in Shreveport, Shreveport, LA 71130 and Departments of Pharmacology and Neurology, Washington University School of Medicine, St. Louis, MO 63110.

There are currently two strains of genetically epilepsy-prone rats (GEPRs), each demonstrating a characteristic pattern of convulsion in response to sound. The moderate seizure animals (GEPR-3s) exhibit a generalized clonic convulsion, whereas the severe seizure animals (GEPR-9s) exhibit a generalized tonic convulsion, each in response to sound. In both strains, wild running is the initial response to sound, preceding the actual convulsion.

Although it is known that GEPRs retain their sound-induced seizure susceptibility for life and that GEPR-3s and GEPR-9s each demonstrate a predictable pattern of convulsion, little is known about the duration of post-ictal refractoriness to sound-induced convulsions in these animals. GEPRs have been sound stimulated once daily for extended periods of time with no decrease in the severity of convulsion. Nonetheless, protocol for anticonvulsant testing in our laboratory requires that GEPRs recover one week from their last sound-induced convulsion before anticonvulsant testing takes place. This minimizes the possibility of confounding post-ictal refractoriness with an anticonvulsant effect. The present study was performed to determine specifically the duration of post-ictal refractoriness of GEPR-3s and GEPR-9s to sound-induced convulsions.

In the present study, GEPRs were sound stimulated twice, with the inter-stimulus interval varying between 0.5 and 24 minutes. Latency to onset of wild running, latency to onset of convulsion and seizure severity were determined at both time points.

Seizure severity returned to normal approximately four times faster in GEPR-3s than in GEPR-9s. Seizure severity was normal within three minutes in GEPR-3s and within twelve minutes in GEPR-9s. In both GEPR-3s and GEPR-9s, latency to onset of wild running took longer to return to normal than seizure severity. Increased latency to onset of convulsion returned to normal in both GEPR-3s and GEPR-9s at a slower rate than any of the other indices of post-ictal refractoriness. (Partially supported by NIH grant NS16829)

384.4 MANNITOL PREVENTS NECROTIC EDEMA ASSOCIATED WITH KAINIC ACID INDUCED SEIZURES. J.P. Olson*, F.E. Samson, T.L. Pazdernik* and S.R. Nelson. Departments of Anatomy, Pharmacology and the Ralph L. Smith Res. Ctr., University of Kansas Medical Center, Kansas City, Kansas 66103.

Repetitive kainic acid (KA) induced seizures in rats produces selective brain damage. Early cellular edema accompanies the seizures and may contribute to the necrosis observed 24 hrs later by interfering with the vascular perfusion of the affected areas as suggested by Sperk et al. *Neurosci.* 10:1301, 1983; Lassman et al. *Neurosci.* 13:691, 1984. Mannitol was infused to determine if the necrotic edema could be prevented by dehydrating the brain during the cellular edema phase. Male Wistar rats (250-325g) were used. Twelve to 24 hrs after cannulation of the femoral vein, the rats were injected with KA (12mg/kg, s.c.) and the ensuing behavior was scored on a 4.0 scale with a 2.0 and above indicating repetitive clonic seizures. At the onset of the clonic seizures (2.0), mannitol (1.6M) or isotonic saline was infused at 0.1ml/min for approximately 30 min. The seizures were stopped with diazepam (5-10mg/kg, i.v.) 60 min after the seizure onset and the rats were sacrificed 24 hrs later for measurement of brain edema (specific gravity change). KA-induced seizures produce significant ($p<0.05$) decreases in specific gravity (necrotic edema) from vehicle treated control in piriform/entorhinal cortex (27% vol increase) and hippocampus (10% vol increase) when infused with saline. No edema was present in thalamus and paricetal cortex. In rats treated with mannitol during the seizure there was no significant edema in any area measured (piriform/entorhinal cortex, hippocampus, thalamus and parietal cortex). Thus, the early cellular edema associated with KA induced seizures appears to play an important role in the later development of regional tissue necrosis.Supported by: U S Army DAMD 17-83-C-3242.

384.5 INCREASE IN THE NUMBER OF HIPPOCAMPAL $^3$H-GLUTAMATE BINDING SITES IN RATS INBRED FOR A GENETIC PREDISPOSITION TO ACOUSTIC STIMULUS INDUCED SEIZURES. S.A. Mills*. C.E. Reigel, P.C. Jobe, and D.D. Savage, Dept. Pharmacol., New Mexico Sch. Med., Albuquerque, NM 87131 and Dept. Basic Sciences, Illinois Col. Med., Peoria, IL 61656 (Spon: R.D. Brown).

Several colonies of genetically epilepsy prone rats (GEPR) have been developed from inbreeding Sprague-Dawley rats exhibiting audiogenic seizures. In response to an acoustic stimulus, rats from the GEPR 9 colony exhibit full tonic extensor seizures, and GEPR 3 rats exhibit running fits followed by clonus. A control colony of rats display no seizure activity. Previous studies have suggested a deficit in monoamine neurotransmission as one factor contributing to the audiogenic seizure responsiveness of GEPR 3 and GEPR 9 rats. Central excitatory neurotransmitter systems have received little study.

The binding of $^3$H-glutamate to a putative glutamate receptor was studied in synaptic membranes prepared from the hippocampal formation (HPF) of controls, GEPR 3, and GEPR 9 rats according to the methods of Werling et. al. (J. Neurochem. 41: 586). At 10 nM $^3$H-glutamate, specific binding was elevated significantly in GEPR 9 rats (0.749 +/- 0.078 pmol/mg prot., n=10) as compared to seizure resistant controls (0.526 +/- 0.065 pmol/mg prot., n=10; $p<0.05$). Specific glutamate binding in GEPR 3 rats was intermediate between that of controls and GEPR 9 rats (0.612 +/- 0.084 pmol/mg prot.). Scatchard analysis of specific binding indicated an increase in total number of binding sites in GEPR 9 rats (19.5 +/- 1.5 pmol/mg prot., n=6) as compared to controls (12.3 +/- 1.1 pmol/mg prot., n=6; $p<0.005$). Total number of binding sites in GEPR 3 rats was 14.8 +/- 3.0 pmol/mg prot. There was no significant difference in the apparent affinity binding constant ($K_d$) in the three groups. These results indicate there is a graded elevation in total number of specific $^3$H-glutamate binding sites in the HPF of GEPR 3 and GEPR 9 rats compared to seizure resistant controls.

GEPR rats have been shown to exhibit kindling stimulus-induced seizures after fewer stimulations than audiogenic seizure resistant controls. Glutamate is thought to be one excitatory neurotransmitter in the HPF. An increase in the number of glutamate receptors in the HPF would suggest a net increase in the excitability of the HPF. An elevation in glutamate binding may explain, in part, why the early stages of kindling are accelerated in GEPR rats. Further, these results indicate that the HPF may be one area contributing to seizure responses of GEPR rats.

384.6 ALTERATIONS IN HIPPOCAMPAL AFTERDISCHARGE ACTIVITY FOLLOWING ADMINISTRATION OF PICROTOXIN, PENTYLENETETRAZOL OR CAFFEINE. L. J. Burdette* & R. S. Dyer. (Spon: L. Grant). Northrop Services, Inc. and U. S. Environmental Protection Agency, Research Triangle Park, NC 27711

Electrical stimulation of the hippocampus results in a train of high amplitude synchronous spike activity, known as the primary afterdischarge (PAD). A period of postictal depression follows and is terminated by the appearance of a second train of spike activity, the rebound afterdischarge (RAD). Although the effects of depressants on hippocampal afterdischarge parameters are well documented, little attention has been directed to the action of stimulants on these measures. The objective of the present investigation was to identify alterations in hippocampal afterdischarge parameters following administration of subconvulsant dosages (half of the minimal convulsant dosage) of picrotoxin, pentylenetetrazol or caffeine.

Long Evans rats (N=104) were implanted with chronic bipolar electrodes in the dorsal hippocampus, with ground and reference screw electrodes placed over ipsilateral and contralateral frontal cortex, respectively. One week later, subjects were assigned randomly to one of four treatment groups: saline (0 mg/kg ip); picrotoxin (2 mg/kg ip); pentylenetetrazol (20 mg/kg ip); or caffeine (75 mg/kg ip). Afterdischarge threshold testing was initiated fifteen minutes after injection. Beginning at 10 μamps, current (2 second train of 50 Hz biphasic pulses) was increased in 10 μamp steps every minute until a PAD was elicited. Afterdischarge threshold, the duration of the PAD, postictal depression and RAD, and frequency of wet dog shakes (WDS) were analyzed.

Hippocampal afterdischarge parameters were affected differentially by the three convulsants. Caffeine dramatically prolonged RAD (threefold increase) without altering PAD duration or other variables. This dissociation between PAD and RAD suggests that different mechanisms may be involved in terminating the two spike trains. Because caffeine has been shown to occupy adenosine receptors at physiological concentrations, adenosine may be implicated in terminating rebound activity in the hippocampus, an adenosine-rich structure. Picrotoxin and pentylenetetrazol decreased WDS frequency; all other parameters remained unchanged. As picrotoxin is a known GABA antagonist, this observation is compatible with evidence that dentate granule cell discharge, inhibited by GABA, is necessary to observe wet dog shakes. Surprisingly, no treatments altered thresholds, or the duration of the PAD or postictal depression relative to control values. Discriminating pharmacological probes may further elucidate mechanisms responsible for hippocampal afterdischarge parameters.

384.7 EFFECT OF NONSTEROIDAL ANTI-INFLAMMATORY DRUGS ON PENICILLIN FOCAL MODEL OF EPILEPSY IN RAT. M.C. Wallenstein.
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Nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit the action of cyclo-oxygenase on arachidonic acid which can lead to production of endoperoxides, prostaglandins or thromboxanes. These metabolites play a role in many functions of the body. A previous study in the rat suggested that this inhibition of CNS arachidonic acid metabolism by NSAIDs decreases and/or blocks the motor component of seizures induced by systemic pentylenetetrazol without having a similar consistent effect on the electrocortical component of the seizures (Wallenstein, 1985). This suggested an "anticonvulsant" action without a concurrent overall anti-CNS excitatory action.

In the present study, this hypothesis was tested on a focal model of epilepsy. Penicillin-G (500 units in 5 μl) was administered to the frontal cortex of conscious, free-moving rats chronically implanted with stainless steel cannula and supracortical screw electrodes. In a series of randomized tests each rat was used as its own control. Penicillin induced CNS excitation was quantified by measuring such parameters as onset latency to, and number of, electrocortical spikes and seizures, myoclonus, and convulsions.

Pretreatment with paracetamol significantly decreased the number of penicillin-induced seizures. Ibuprofen significantly decreased the number, and delayed onset, of electrocortical seizures. Mefenamic acid significantly delayed onset, but increased the number, of electrocortical seizures. However, all of these NSAIDs significantly decreased the number of motor seizures (i.e. convulsions). These results are consistent with the hypothesis that the NSAID induced effect on motor seizures results from inhibition of arachidonic acid metabolism while the drug-specific actions result from either tissue-specificity of NSAIDs (Flower and Vane, 1974) or from actions not related to cyclo-oxygenase inhibition (Collier and Sweatman, 1968; Levy and Lindner, 1971; Phillis and Wu, 1981).

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384.8 EFFECT OF PRECOLLICULAR TRANSECTION ON ELECTROSHOCK AND PENTYLENETETRAZOL-INDUCED SEIZURES IN RATS. R.A. Browning and D.K. Nelson*. Southern Illinois University, School of Medicine, Carbondale, IL 62901.

The motor components of experimentally-induced seizures (convulsions) have long been used to assess seizure severity and to evaluate potential antiepileptic drugs. Convulsions in experimental animals generally consist of clonic and/or tonic components. We have recently suggested that clonic convulsions in rats can be divided into at least two subtypes: (1) face and forelimb (F&F) clonus which is seen in the early stages of pentylenetetrazol (PTZ) seizures as well as in minimal electroshock seizures and (2) running-bouncing (R/B) clonus which is observed in the latter stages of PTZ seizures and in rats undergoing minimal audiogenic seizures.

Previous studies have shown that tonic convulsions and R/B clonus are suppressed by lesions in the pontine reticular formation, while clonus is not. Therefore, we have proposed that both tonic convulsions and R/B clonus require a neural substrate in the brainstem for expression, while F&F clonus depends on a discharge emanating from the forebrain. In order to test this hypothesis further, we have examined the effect of a precollicular transection on convulsions induced by minimal electroshock (a.c.EST), maximal electroshock (MES) and PTZ.

Brain transections (between the diencephalon & midbrain) were made in male Sprague-Dawley rats (260-360g) under ether anesthesia and seizure testing was carried out 3 hours after transection or sham-surgery. Sham-operated rats treated with PTZ (50 mg/Kg i.p.) displayed the following sequential convulsive pattern: F&F clonus → twisting clonus → R/B clonus → tonus, while transected rats displayed only R/B clonus → tonus. Similarly, the transection was found to abolish F&F clonus in rats receiving a.c. EST., although R/B clonus or tonus could still be elicited when higher stimulating currents were employed. Transected rats subjected to the usual MES stimulus (150 mA, 0.2 sec.) exhibited tonic flexion, but failed to display hindlimb extension. However, transected rats subjected to higher stimulus MES (200 mA, 0.4 sec.) displayed a typical maximal convulsion consisting of tonic flexion, tonic hindlimb extension and clonus.

These findings support the hypothesis that tonic and some types of clonic convulsions can occur independently of seizure discharge in forebrain structures, while clonus restricted to the face and forelimbs seems to depend on structures within the forebrain for expression.

384.9 ABNORMALITIES IN BRAIN NOREPINEPHRINE LEVELS IN GENETICALLY EPILEPSY-PRONE RATS. J.W. Dailey, C.E. Reigel, Jr.*, K.H. Ko*, M.T. Acurio*, J.E. Penny and P.C. Jobe. Depts. of Pharmacol., Psy. and Anat., LSUMC, Shreveport, LA 71130, Dept. of Pharmacol., Sch. of Pharm., Seoul Natl. Univ., Seoul, Korea and Dept. of Basic Sci., Univ. of IL, Coll. of Med., Peoria, IL 61656.

The seizure prone-state of the genetically epilepsy-prone rat (GEPR) is characterized by susceptibility to sound-induced seizures and hyperthermic seizures. Some GEPRs also appear to experience spontaneous seizures and dolorogenic seizures. In addition, the GEPR exhibits abnormal responses to electroshock, pentylenetetrazole, bicuculline and barbiturate withdrawal seizures. We have previously shown (Biochem. Pharmacol. 30:3157, 1981) that manipulations that decrease synaptic norepinephrine increase seizure severity whereas manipulations that increase synaptic norepinephrine decrease seizure severity in the GEPR. We have also reported (Fed. Proc. 43:2505, 1984) that there are widespread deficits in brain norepinephrine concentration and turnover rate in the GEPR. These studies showed that at least two types of brain norepinephrine deficits exist. One type of deficit is graded in that moderate seizure GEPRs (GEPR3) have moderate decrements in norepinephrine concentration and severe seizure GEPRs (GEPR9) have severe decrements in norepinephrine concentration. In the other type of deficit, the decrements in norepinephrine are of equal magnitude in both moderate (GEPR3) and severe (GEPR9) seizure animals. Because of these and other data we have hypothesized that deficits in brain norepinephrine partially regulate both seizure severity and seizure susceptibility in the GEPR.

We are now carrying out studies designed to trace the noradrenergic deficits to smaller and smaller brain areas and to determine in which areas deficits are graded and in which areas they are not. For this study we examined norepinephrine concentration in six brain areas of animals whose seizure characteristics had been confirmed by testing. That is, each animal had been exposed to an acoustic stimulus three times at weekly intervals. In each of the three tests each animal experienced its characteristic convulsion. The brain areas examined were inferior colliculus, superior colliculus, medulla, midbrain tegmentum, basal pons and pons tegmentum. In each of these areas GEPRs exhibited abnormally low brain norepinephrine concentrations when compared with control. None of the areas had graded deficits. That is, severe seizure animals (GEPR9) did not have greater deficits than moderate seizure animals (GEPR3). We conclude that norepinephrine in these brain areas may regulate seizure susceptibility but not seizure severity in the GEPR. (Supported in part by NS16829.)

384.10 ABNORMAL BRAIN 5-HYDROXYTRYPTAMINE LEVELS IN GENETICALLY EPILEPSY-PRONE RATS. P.C. Jobe, C.E. Reigel, Jr.*, K.H. Ko*, M.T. Acurio*, J.E. Penny and J.W. Dailey (SPON: John J. Stewart). Depts. of Pharmacol., Psy. and Anat., LSUMC, Shreveport, LA 71130, Dept. of Pharmacol., Sch. of Pharm., Seoul Natl. Univ., Seoul, Korea and Dept. of Basic Sci., Univ. of IL, Coll. of Med., Peoria, IL 61656.

The genetically epilepsy-prone rat (GEPR) is susceptible to sound-induced seizures and to hyperthermic seizures. These animals also exhibit abnormal sensitivities to electroshock and several of the chemical convulsants. Presently there are two colonies of seizure susceptible GEPRs maintained at the University of Illinois College of Medicine in Peoria. One colony is composed of moderate seizure animals (GEPR3) that experience a wild running phase that terminates in generalized clonus when sound-stimulated. The second colony is composed of severe seizure animals (GEPR9) that experience a wild running phase that terminates in a tonic extensor convulsion when sound stimulated. A colony of seizure resistant controls is also maintained. The breeders used in the control colony are tested and are known to be resistant to sound-induced seizures.

Pharmacologic studies have suggested that brain 5-hydroxytryptamine may play an important role in regulating seizure susceptibility and severity in the GEPR. Also, previous pathophysiologic studies in large brain areas have demonstrated widespread abnormalities in brain concentrations of 5-hydroxytryptamine.

For the present report we dissected the brain into smaller areas in order to begin to trace the deficits in 5-hydroxytryptamine to specific tracts. In this study we determined 5-hydroxytryptamine levels in 6 brain areas of animals that had been sound-stimulated and had experienced convulsions. In the medulla, midbrain tegmentum, superior colliculus and pons tegmentum 5-hydroxytryptamine levels were significantly lower than control in moderate seizure (GEPR3) and severe seizure (GEPR9) animals. These deficits were not greater in the severe seizure animals than in the moderate seizure animals. That is, they were not graded. In the basal pons and the inferior colliculus the 5-hydroxytryptamine levels in moderate seizure (GEPR3) animals were significantly lower than control and significantly lower than the levels in severe seizure (GEPR9) animals. The levels in severe seizure animals did not differ from control. These results suggest that abnormalities in 5-hydroxytryptamine concentration in the medulla, midbrain tegmentum, superior colliculus and pons tegmentum may play a role in the regulation of seizure susceptibility in the GEPR. Because the severe seizure (GEPR9) animals did not have lower 5-hydroxytryptamine levels than control, the data do not suggest a similar role for this biogenic amine in the inferior colliculus and basal pons (supported in part by NS 16829).

384.11 DIFFERENCES BETWEEN STRYCHNINE AND PENICILLIN EPILEPTOGENESIS SUGGEST A LAMINAR ORGANIZATION OF NEOCORTICAL INHIBITION. A.B. Chatt and J.S. Eberaole. Department of Neurology, Yale University School of Medicine, New Haven, CT 06510 and Epilepay Center, VA Medical Center, West Haven, CT 06516.

We have demonstrated recently that stellate layer 4 of cat striate cortex is the lamina most susceptible to the epileptogenic effects of PENICILLIN, a partial blocker of GABA mediated inhibition; while superficial pyramidal layers 2 and 3 are uniquely sensitive to the epileptogenic effects of STRYCHNINE, a putative blocker of GLYCINE mediated inhibition. Based on this very distinctive tissue segregation of convulsant sensitivities, we have concluded that laminar differences in inhibitory mechanisms may exist in cat visual cortex. In this report, we present evidence that these two convulsants appear to differentially influence extrinsically (thalamocortical) and intrinsically (local circuit, LCN) evoked cortical activity.

Epileptogenesis, in our model, proceeds through two distinct stages before large negative epileptiform potentials are recorded simultaneously from each cortical layer. The first stage is the enhanced amplitude of the normal (physiologic) evoked response (EPR) which has been shown to be a result of extrinsic, thalamocortical afferent barrages. The second is the late response (LR) occurring later in time than the EPR and believed to be the result of intrinsic/ LCN activity.

Regardless of the lamina injected, we have never seen PENICILLIN epileptogenesis without an initial EPR in layer 4, site of the major thalamic input to the cortex; making penicillin's action in this regard critical to the onset of epileptiform abnormalities in all cortical layers. Autoradiographic evidence indicates that $^{14}C$-labeled penicillin in contact with layer 4 may be critical to the generation of these EPRs. STRYCHNINE's epileptogenic action, however, appears to be independent of both layer 4 and the extrinsically evoked EPR. Only injections into the superficial pyramidal layers, where extrinsic influences are considerably reduced, ellicited epileptiform activity reliably; with LRs occurring there without prior EPRs in layer 4. Injection directly into layer 4 seldom induced multilaminar epileptiform alterations, but when it did, this activity occurred very late (4-6 min, post-injection) there and simultaneously in the superficial layers as an intrinsically-mediated LR; perhaps indicating that strychnine diffusion into the most sensitve superficial layers is necessary to initiate epileptogenic alterations.

STRYCHNINE, therefore, exerts it's epileptogenic action principally where direct thalamic driving is minimal and it's influence can most easily be seen on LCN-mediated cortical potentials. PENICILLIN's principal action is on thalamically evoked cortical activity.

384.12 **CORRELATIONS BETWEEN ANTICONVULSANT ACTIVITIES WITH PLASMA AND BRAIN CONCENTRATIONS OF AHR-11748, A NEW ANTIEPILEPTIC.** M. A. Osman*, L. K. Cheng*, D. N. Johnson, and G. J. Wright*. (SPON: J. A. Rosecrans). A. H. Robins Research Lab, Richmond, VA 23261-6609.

In order to correlate the anticonvulsant effects of AHR-11748, (3-[3-(trifluoromethyl)phenoxy]-1-azetidinecarboxamide), with its concentrations in the plasma and brain, groups of 16 mice received intraperitoneal doses ranging from 10 to 225 mg/kg. Thirty min after dosing, half of the mice in each group were subjected either to maximal electroshock (ES) or Metrazol (M) challenge. The other animals were sacrificed by decapitation, and plasma and brain samples were collected and pooled (n = 4). Concentrations of AHR-11748 were determined by a specific HPLC method. The results showed that the plasma and brain concentrations of AHR-11748 were directly proportional to the doses administered. The concentrations in the brain were consistently higher than those in plasma; the tissue-to-plasma ratio was 3.4 ± .38 over the entire dose range. When the concentration of AHR-11748 was 48.0 µg/g in the brain (14.5 µg/mL in plasma), then 50% protection against ES was observed; 77.0 µg/g in the brain (21.4 µg/mL in plasma) was correlated with 100% protection. The brain and plasma concentrations were about 3.5 times higher to provide corresponding levels of protection after M challenge. The duration of action (protection against ES) of AHR-11748 was determined in mice dosed with 60 mg/kg of AHR-11748 intraperitoneally. Total protection was observed up to 2 hr after dosing and the effect was reduced to 10% after 4 hr. The decline in pharmacologic effects correlated with to the elimination profiles of AHR-11748 in the plasma and brain.

384.13 **AHR-11748, A POTENTIAL ANTIEPILEPTIC AGENT.** David N. Johnson, Brian F. Kilpatrick,* and Ewart A. Swinyard,* A. H. Robins Research Labs, Richmond, VA 23261 and U. Utah, Salt Lake City, UT 84112.

AHR-11748 (3-[3-(trifluoromethyl)phenoxy]-1-azetidinecarboxamide) was effective in mice and rats in preventing seizures induced by electrical (maximal electroshock) and chemical (sc Metrazol, bicuculline, picrotoxin) challenge. The pharmacologic profile of activity of AHR-11748 most closely resembled that of phenobarbital and valproic acid, and differed from that of phenytoin and ethosuximide. The potency of AHR-11748, administered IP, was approximately 1/2 to 1/5 that of phenobarbital; compared with valproic acid, AHR-11748 was approximately 2 to 6 times more potent. The drug was well absorbed orally; the oral $ED_{50}$s were approximately 1 1/2 times greater than the intraperitoneal $ED_{50}$s. Tolerance to the anticonvulsant effects did not develop in rats when the drug was administered daily for 5 days. In concentrations up to $10^{-4}$M, AHR-11748 did not displace or enhance $^{3}$H-GABA from mouse whole brain synaptosomes, nor did it displace or enhance $^{3}$H-flunitrazepam from rat cortex synaptosomes. Further, AHR-11748, at $10^{-4}$M, did not displace $^{35}$S-TBPS binding from rat whole brain synaptosomes. Behavioral effects in mice after large doses of AHR-11748 included ataxia, muscle weakness, and decreased spontaneous motor activity. The $ED_{50}$s for the rotorod and the loss of righting reflex tests were 203 and 413 mg/kg, IP, respectively. AHR-11748 was relatively weak in blocking morphine-induced Straub tail in mice; its $ED_{50}$, 35 mg/kg, IP, was approximately 50 times greater than that of diazepam. AHR-11748, at 5 mg/kg, IV, produced a 35% suppression of the polysynaptic linguomandibular reflex with essentially no effect on the monosynaptic patellar reflex in cats. These data suggest that AHR-11748 is a potential antiepileptic agent without marked central muscle relaxant properties. (Supported, in part, by contract number NO. 1-NS-4-2361 from the Epilepsy Branch, National Institute of Neurological and Communicative Disorders and Stroke [NINCDS], NIH, to Dr. Swinyard.)

384.14 SEIZURE INDUCTION BY FOCAL INFUSION OF EXCITANT AMINO ACIDS INTO THE INFERIOR COLLICULUS AND SUPERIOR COLLICULUS. B.S. Meldrum*, M.H. Millan* and C.L. Faingold. Dept. of Neurology, Institute of Psychiatry, London SE5 8AF UK and Dept. Pharmacology, Southern Illinois Univ. Sch. of Med., Springfield, IL 62708 USA.

The inferior colliculus (IC) is reported to play an important role in audiogenic seizures (AGS) in the Genetically Epilepsy Prone (GEP) rat. Electrical and chemical stimulation of the IC produce wild running seizures (WRS) [6] which are a major component of AGS in the GEP rat. AGS can be blocked by infusion of an excitant amino acid antagonist into the IC of GEP rats [4]. Recent studies [5] also suggest that an excitant amino acid may be a normal neurotransmitter in the IC, and elevation of aspartate levels is observed in the IC of GEP rats during AGS [2].

This study examined if seizure could be induced by infusion of N-methyl-D-aspartate (NMDA). Guide cannulae were implanted in normal rats over IC or superior colliculus (SC). At least one week later 10-20 nM of NMDA in 0.25-0.5 µl of phosphate buffer was infused (over 4 min) bilaterally. The animals were tested for AGS (electrical bell at 109 db) and observed for spontaneous WRS. All of the 18 rats injected in IC exhibited seizures, 39% showed AGS only, while 61% showed spontaneous WRS and AGS. Of the 15 rats injected in SC, 60% did not exhibit seizures, 32% showed only spontaneous WRS, and only one exhibited AGS. Vehicle infusion alone was ineffective. Preliminary data indicated that infusion of excitant amino acid into reticular formation (RF) did not cause AGS, although spontaneous seizures were sometimes observed.

These data, along with the proposed action of excitant amino acids in normal neurotransmission in IC [4] and the elevation of aspartate levels in IC during AGS [2] as well as the blockade of AGS by an excitant amino antagonist injected into IC [4] suggest that amplified release of an excitant amino acid in IC may be an important mechanism in the etiology of genetic susceptibility to AGS. The efficacy of excitant amino acid infusion in IC contrasts with the ineffectiveness in SC and RF and suggests the relatively specific involvement of IC in induction of AGS susceptibility. The induction of spontaneous WRS by infusion into the SC may activate the proposed seizure pathway which involves the substantia nigra [3]. This may be one of the output pathways along with those of the RF [1] which mediate the components of AGS. However, activation of these pathways by acoustic stimuli may require input from auditory nuclei primarily via the IC.

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384.15 ACCUMULATION OF FREE ARACHIDONIC ACID AND LIPOXYGENASE REACTION PRODUCTS FOLLOWING BICUCULLINE-INDUCED STATUS EPILEPTICUS IS LOCALIZED IN RAT CEREBRAL SYNAPTOSOMES. Nicolas G. Bazan and Dale L. Birkle*. Louisiana State University Medical Center, LSU Eye Center, New Orleans, LA 70112.

Free fatty acid (FFA) accumulation is one of the earliest detectable biochemical events after stimulation of the central nervous system, e.g. electroconvulsive shock and ischemia. Accumulation of FFA may be the result of an overstimulation of mechanisms for membrane tailoring, that operate normally during neurotransmission. To test this we have studied the metabolism of the major accumulated FFA, arachidonic acid (20:4, n-6) in rat cerebral synaptosomes and microsomes following bicuculline-induced status epilepticus. Rat brain was prelabeled in vivo by the bilateral, intraventricular injection of 1 µCi [1-$^{14}$C]20:4. Thirty min later, rats were intubated, paralyzed with tubocurarine and placed on mechanical ventilation. Scalp electrodes were attached for monitoring of the EEG. One hr after injection of 20:4, rats were treated with bicuculline (10 mg/kg, ip.) and killed by decapitation during the status epilepticus phase, as assessed by EEG. Lipids were extracted from the subcellular fractions immediately upon isolation, or the fractions were incubated at 37° C for 60 min in the presence or absence of 45 mM $K^+$. Analysis of labeling of prostaglandins (PGs) and lipoxygenase reaction products (HETEs and leukotrienes) were done by high performance liquid chromatography. The identity of HETEs was confirmed by gas chromatography-mass spectrometry. In addition the endogenous FFA levels were measured. In control groups the level of FFA was high, due to release during subcellular fractionation; however a 25% increase in 20:4 was detectable in synaptosomes from bicuculline-treated animals. There were no detectable changes in FFA levels in the microsomal fraction. We detected four lipoxygenase reaction products in the synaptosomal and microsomal fractions. The major products in synaptosomes were 12-HETE and $LTB_4$ and in microsomes, $LTB_4$ and a mono-HETE (as yet identified but not 15-HETE, 5-HETE or 12-HETE). All of the major PGs were detected in both fractions, with higher activity in microsomes. Bicuculline treatment elevated lipoxygenase reaction products in synaptosomes and microsomes, although the profile of products was differentially stimulated. PGs were increased in microsomes, and decreased in synaptosomes. In incubated synaptosomes there was a marked stimulation of $PGD_2$, which was more pronounced in bicuculline treated animals. Depolarization by $K^+$ caused effects similar to bicuculline in synaptosomes, and had no effect in microsomes. The results indicate that bicuculline-induced seizures cause the release of 20:4 from synaptic membranes and increase the production of eicosanoids in synaptosomes.

384.16 AUDIODESENSITIZATION INDUCES AN ENDOGENOUS ANTICONVULSANT SYSTEM THAT PREVENTS SPONTANEOUS SEIZURES IN GERBILS AND HAMSTERS. Paul L. Prather* and W. B. Iturrian. Pharmacology Dept., Univ. Georgia, Athens, Ga. 30602

In epileptics, a postictal phase prevents a second seizure for varying intervals - occasionally lasting days. The neurological mechanisms are obscure but activation of endogenous anticonvulsant system(s) is an attractive possibility with profound implications. We are attempting to study the chemical basis for such long term anticonvulsant effects and demonstrate protection from the hereditary spontaneous seizures (SS) of gerbils and hamsters using a nonconvulsive technique - Audio-desensitization (ADS).

Gerbils that are genetically epileptic have brief tonic-clonic seizures which are elicited by vague stimuli such as handling but become refractory for a postical period lasting 2 or 3 days. In contrast the spontaneous seizure of the sz hamster (Bio 86.93) persists for 2 or 3 hours without an apparent postictal protective phase. Sound does not precipitate the seizure in either species.

LVK hamsters can be audiosensitized for sound induced seizure susceptibility at 28-31 days of age (Iturrian and Johnson, Experientia 27: 1193, 1971). Audiogenic seizures only occur 14 to 21 days later and 60 sec. sound exposures (audiodesensitization) at 3 day intervals prevents SS completely. ADS apparently acts through the induction of an endogenous anticonvulsant protein (Fed. Proc., 41: 1073, 1982).

We now demonstrate that this same audiodesensitization regimen protects sz hamsters from spontaneous or pentylenetetrazol induced seizure. Although ADS resulted in a less dramatic reduction in the incidence of spontaneous seizure in the gerbil it reproducibly causes a 50% reduction in seizure incidence. The regimen of ADS is very important. Surprisingly a single 316 sec (95 dbA) sound blocked postictal depression allowing gerbils to convulse twice within a 3 hr. period; although only 22% seizured 7 days later, in contrast to the 64% observed in controls. ADS is blocked by ether or cycloheximide when administered during each of the desensitization regimen exposures suggesting a protein dependent memory-like process.

384.17 A Chronic Feline Model of Limbic Epilepsy. R. G. Fariello, G.T. Golden, P.F. Reyes*, G.G. Smith*.Neurology and Research, VAMC Coatesville PA 19320 and Thomas Jeff. Med. College, Phila. PA 19107

Epilepsy research is handicapped by the lack of suitable inexpensive, durable animal models. Injection of cobalt powder in the hippocampus or amygdala of cats provides 45 - 60 days of epilepsy. Intralimbic injection of alumina gel gives rise to a longer lasting model but the method of induction does not allow exact quantification of the technique and destruction of small anatomical structures occur prior to the generation of epileptic activity.

Since several medical intractable human limbic epilepsies are multifocal in nature, we have investigated the effect of micro injections of 3.8 µl aqueous solution of cobalt chloride in the hippocampus and amygdala of the same experimental animal. Three cats received this treatment under general anesthesia,thereafter EEG and behavior were monitored on a daily basis. One cat received injections of equal volume and molarity of ferric chloride. Of the cobalt animals two were sacrificed at one month and one after eight months; the $FeCl_3$ cat is still alive after six months. All the cobalt animals were having active electro-behavioral seizures and interictal activity at the time of sacrifice. The following types of seizures were observed: 1) infraclinical, 2) partial complex with or without secondary generalization, 3) convulsive tonic clonic status epilepticus. The $FeCl_3$ cat did show transient interictal spiking but to this day has not developed any ictal discharges. No anticonvulsive treatment was attempted except for administration of diazepam and phenytoin acutely in 4 episodes of generalized tonic clonic status epilepticus which occurred in two animals, three times in one cat at one,four and six months, one time in another cat ,four weeks post cobalt. The interaction between foci, the onset and spread of interictal and ictal activity, the involvement of contralateral structures and of the Substantia Nigra will be illustrated. In two animals ipsilateral Nigral stimulation consistently triggered ictal discharges mostly but not exclusively arising from the hippocampus with sudden spread to other limbic structures, electro-clinical seizures and often secondary generalization. The histopathological features of the model will also be described. These preliminary results are encouraging and ongoing experimentation is aimed at refining this potential model of perhaps permanent epilepsy in cat.

## DISEASES OF THE NERVOUS SYSTEM: EPILEPSY, KINDLING

385.1 IBOTENIC ACID-INDUCED LESIONS OF THE HIPPOCAMPUS FACILITATE AMYGDALA KINDLING IN RATS. D.D. Walczak*, L.E. Jarrard, and J.L. Meyerhoff (SPON: F.J. Manning) Dept of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, D.C. 20307.

In order to study the importance of the hippocampus in the kindling process in the rat, the hippocampus (CA1 - CA3, dentate gyrus) was lesioned with multiple injections of ibotenic acid (IBO), a procedure that destroys cells while sparing fibers-of passage. Control animals received lowering of the needle at the same sites as the hippocampals (sham-operated controls). A mixture of pentobarbital and chloral hydrate was used for surgical anaesthesia. Immediately after injections of IBO, stainless steel bipolar electrodes were implanted in the left amygdala, and stainless steel screw electrodes were placed over the frontal cortex. Animals were allowed to recover for two weeks prior to the initiation of kindling. Kindling stimuli (left amygdala, 1 sec train of 1 msec biphasic pulses, 60 Hz, 200 uA base-peak) were given daily. Cortical and amygdala EEG, and behavioral convulsions were recorded after each stimulus. Histological analysis of the resulting lesions (cell stain and Fink-Heimer silver stain) indicated extensive damage to both dorsal and ventral hippocampus with the only sparing being limited to small areas in the posterodorsal dentate gyrus and CA1 cell field.

The cumulative afterdischarge measured in drug-naive and drug treated rats is a sensitive indicator of changes in CNS excitability. Facilitation of the development of motor seizures is defined in this study as a drop in the amount of cumulative afterdischarge required to elicit stage 5 seizures. Inhibition is defined as an increase in cumulative AD to reach stage 5. Lesions produced no significant changes in latency to kindle to stage 3 or to stage 5, and no significant differences in daily seizure score. However, daily afterdischarge duration was significantly lower ($p = 0.0503$), and the cumulative afterdischarge duration was lower in lesioned rats (481.1 sec + 80.6 lesion vs. 711.2 sec + 97.4 sham). Thus, lesioned animals kindled at the same rate as the sham-lesioned controls, but did so with less elicited epileptiform activity. This facilitation of the kindling process in lesioned rats raises new questions about the role of the hippocampus in the development of amygdala kindled seizures.

385.2 FUNCTIONAL 2-DEOXYGLUCOSE MAPPING OF PROGRESSIVE STATES OF STATUS EPILEPTICUS INDUCED BY AMYGDALA STIMULATION IN RAT. A. Handforth* and R. Ackermann (SPON: J. Engel, Jr.) Dept. of Neurology, Div. of Nuclear Medicine and Biophysics, UCLA School of Medicine, Los Angeles CA 90024.

Delivery at 2 Hz of short trains of bipolar pulses to the amygdala for 20-50 minutes via implanted electrodes has been found by us reliably to elicit status epilepticus (SE) which is self-sustaining for hours and does not require prior kindling. In this model several distinct states of SE may be elicited, which differ in severity. The least severe state consists of continuous non-habituating exploration (ambulatory SE). Next in severity is a state of continuous mastication (class 1 SE). More severe is a state of vigorous nodding, rearing, and frequent clonus (class 2+ SE).

Quantitative assessment of glucose metabolism in each of these states was conducted using $^{14}$C-2-deoxyglucose (2DG) autoradiography. The 2DG was injected at least 20 minutes after SE induction. The most common pattern associated with ambulatory SE was unilaterally increased 2DG uptake indicative of activation in limbic and related structures. These included amygdala, hippocampus, subiculum, entorhinal cortex, olfactory areas, septal nuclei, bed nucleus of stria terminalis, dorsomedial thalamus, nucleus accumbens, medial prefrontal cortex, and part of substantia nigra. Variable bilateral involvement of amygdala, hippocampus, substantia nigra and dorsomedial thalamus was also noted. A less common pattern seen in ambulatory SE was considerably more restricted, involving the amygdala, dorsomedial thalamus, bed nucleus of stria terminalis, and medial prefrontal cortex. The pattern corresponding to class 1 SE was a bilateral representation of the more common ambulatory SE pattern. In class 2+ SE there was, in addition, recruitment of the neocortex (especially prefrontal), caudate-putamen, motor thalamic nuclei and substantia nigra.

These results suggest that a small number of nuclei participate in the earliest states of sustained seizure discharge induced by amygdala stimulation. With seizure progression more limbic-related structures are involved, at first ipsilaterally, then bilaterally. As clonus is attained, more neocortical areas and subcortical motor nuclei are recruited.

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385.3 DEVELOPMENT OF A COMPUTER AUTOMATED KINDLING AND EEG MONITORING DEVICE FOR THE STUDY OF KINDLING STIMULUS-INDUCED SPONTANEOUS SEIZURES. C. Gage*, P. Madden* and D.D. Savage. Dept. Pharmacol., Univ. New Mexico Sch. Med., Albuquerque, N.M. 87131.

Kindling is a phenomenon in which repeated administration of a subconvulsive dose of electrical current leads to the gradual development of limbic and motor seizures. Kindling has been proposed as a model of limbic epilepsy. While kindled animals have a permanent susceptibility to stimulus-induced seizures, they rarely display spontaneous EEG or motor seizure behavior. Further, kindling stimulus-induced seizures are relatively unaffected by agents approved for treatment of limbic epilepsy.

Continued application of kindling stimulations well beyond the number required to "kindle" an animal can lead to the occurrence of spontaneous limbic and motor seizure activity. Phenytoin, which has little effect against kindling stimulus-induced seizures, reduces the number of spontaneous seizures in animals kindled to the spontaneous seizure state. The production of animals kindled to spontaneous seizures by manual methods is difficult and time consuming. A computer assisted system has been built to stimulate rats on a programmed schedule and monitor EEG activity during kindling stimulations and interstimulus intervals.

Bipolar electrodes are implanted into the right angular bundle of the entorhinal cortex and the left basolateral nucleus of the amygdala of Sprague-Dawley rats. One week later, rats are placed in chronic EEG recording cages and kindling stimulations given eight times a day at three hour intervals. Between stimulations, the number of EEG spikes are counted by computer and spike frequency analyzed every two seconds. When spike frequency criteria are satisfied for two adjacent sampling intervals, EEG activity is recorded on paper. EEG tracings are analyzed daily for evidence of spontaneous electrographic seizure activity. Animals are observed at least one hour a day during weekdays for evidence of spontaneous motor seizure behavior.

Several preliminary observations have been made using this system: 1.) Class 4 angular bundle kindled motor seizures occur with fewer stimulations in animals stimulated around the clock as compared to three times a day during daylight hours. 2.) Spontaneous electrographic seizure activity in amygdala is observed after 60 to 80 stimulations. 3.) Spontaneous motor seizures have been observed in all stimulated animals. These spontaneous motor seizures resemble motor seizures observed with kindling stimulations, ranging from Class 0 to mild Class 4 types. 4.) After 150 stimulations, the stimulations were discontinued and the animals monitored for four weeks. Both electrographic and motor seizure responses were still present at the end of four weeks. 5.) This computer assisted kindling and EEG monitoring system appears useful for the production and subsequent study of animals exhibiting spontaneous seizures.

385.4 THE DEVELOPMENT OF ANGULAR BUNDLE KINDLED SEIZURES IS ACCELERATED IN RATS WITH A GENETIC PREDISPOSITION TO AUDIOGENIC SEIZURES. D.D. Savage, C.E. Reigel and P.C. Jobe. Dept. Pharmacol., U. New Mexico Sch. Med., Albuquerque, NM 87131 and Dept. Basic Sci., U. Illinois Sch. Med., Peoria, Ill., 61656 (Spon: H.E. Laird).

Experiments were undertaken to examine the kindling phenomenon in three colonies of Sprague-Dawley rats inbred for characteristic seizure responses induced by an acoustic stimulus. In response to an acoustic stimulus, rats from a GEPR-9 colony exhibit running fits followed by full tonic extensor seizures. GEPR-3 colony rats exhibit running fits followed by clonus and rats from the GEPR-0 colony display no seizure behavior in response to an acoustic stimulus. Previous studies have suggested that deficits in monoamine neurotransmission are one factor contributing to the audiogenic seizure responses of GEPR-3 and GEPR-9 rats. Recently, graded elevations in the number of glutamate receptors in hippocampal formation of GEPR-3 and GEPR-9 have been observed.

A bipolar electrode was implanted in the right angular bundle of the entorhinal cortex of male rats from each of the three colonies. One week later, kindling stimulations (700 microampere biphasic square wave pulses, one millisecond in duration at 60 Hz. for two seconds) were administered three times a day at minimum intervals of four hours. Motor seizure behavior in response to the kindling stimulation was scored using the seizure classifications of Racine (1972). After exhibiting three Class 5 motor seizures, the rats were killed and electrode placement verified histologically.

The mean total number of kindling stimulations required for each colony to exhibit three motor seizures of each seizure class was determined. The results indicated that the early stage of kindling development (Class 0 to 1) was significantly accelerated in both the GEPR-9 and GEPR-3 rats, compared to the GEPR-0 rats. The middle stages of kindling development (Class 1 to 3) and the later stages (Class 3 to Class 5) were accelerated to GEPR-9 but not GEPR-3 rats, as compared to the audiogenic seizure resistant controls. Thus a differential and graded acceleration of kindling development was exhibited by GEPR-3 and GEPR-9 rats.

These results suggest the possibility that some brain area(s) involved in the early stages of kindling development may have an altered excitability in both GEPR-3 and GEPR-9 animals. In addition, some other brain area(s) involved in the later stages of kindling development may be altered in GEPR-9 rats also. Alternatively, some brain area(s) responsible for an acceleration of the early stages of kindling may be altered to a greater degree in GEPR 9 than GEPR-3 rats such that later stages of kindling development are facilitated. These putative alterations may, in part, underlie the differential seizure responses of GEPR-3 and GEPR-9 rats to an acoustic stimulus.

385.5 **FREQUENCY DEPENDENT INITIATION OF KINDLED SEIZURES.** **J.H. Goodman*, R.W. Homan, and I.L. Crawford. Dept. of Neurology, University Texas Health Science Center at Dallas and Epilepsy Research Center, VAMC, Dallas.**

**The role of current intensity in the generation of kindled seizures has been studied extensively. However, the dependence of the initiation of kindled seizures on stimulus frequency has not been well characterized. We examined the effect of different stimulus frequencies on eliciting seizures in amygdaloid kindled rats. Adult male Sprague-Dawley rats were bilaterally implanted with electrodes in the basolateral amygdalae. Each animal was stimulated once a day for one second with 400 uA, 60 Hz bipolar, square wave pulses (0.5 msec duration) until three consecutive Stage V seizures occurred. At this time the animal was considered fully kindled. The following stimulus paradigm was used to test: (1) whether Stage V kindled seizures could be elicited by stimulating at frequencies less than 60 Hz, (2) to determine the minimal frequency required to elicit a kindled seizure. Each animal was stimulated once daily at a given test frequency for 60 seconds or until a Stage II seizure was observed. The number of stimuli delivered, the time to reach Stage II and whether a Stage V seizure was attained were recorded. The frequencies tested ranged from 1 to 10 Hz with all other stimulus parameters kept constant. We observed that Stage V seizures could be elicited with stimulus frequencies less than 60 Hz. The minimum frequency which caused a Stage V seizure (5 of 8 animals) was 3 Hz; the apparent threshold frequency under these conditions. At frequencies greater than 3 Hz all animals had a Stage V seizure. For animals stimulated at 5 Hz the mean (±SEM) number of stimuli delivered to each animal ranged from 80.3±11.8 to 224.3±41.3. The average time for each animal to Stage II ranged from 9.8±0.8 to 44.9±8.3 seconds. Increasing frequency of stimuli was inversly related to the number of stimuli necessary to evoke a seizure; however, the time to a Stage II decreased dramatically. For example, at 7 Hz the time to Stage II ranged from 8.7±1.2 to 31.9±2.3.**

**These results demonstrate that frequency can be a factor in the ability to elicit kindled seizures. The utility of using frequencies less than 60 Hz is that one can expand the time to elicit a seizure and it may possibly provide a more sensitive index of seizure threshold.**

385.6 ACTIVATION OF MULTIPLE UNIT ACTIVITY IN THE LOCUS COERULEUS PRODUCED BY AMYGDALA KINDLING. Carlos A. Jimenez-Rivera*, Gerald K. Weiss* and Anna Maria Voltura*. (SPON G. Wild). Department of Physiology, University of New Mexico, School of Medicine, Albuquerque, New Mexico 87131.

Activation of the noradrenergic (NA) system of the rat brain by stimulating the locus coeruleus (LC) lengthens only the early stages in the development of amygdala kindled seizures while lesions of the ascending LC projections accelerates the process. We have hypothesized that activation of the LC during the early stages (1&2) of amygdala kindling contributes to the normal delay of these stages. This activation is produced by the afterdischarges (AD) that occur following an amygdala kindling stimulation.

We designed experiments to record multiple unit activity from the LC during the development of amygdala kindled seizures. Sprague-Dawley rats were implanted with small, monopolar electrodes in the LC and one bipolar amygdala electrode. The protocol consisted of amygdaloid stimulation three times per day at 90 min. intervals (400 u Amp, 1 msec, 60HZ) until 3 consecutive stage 5 were achieved.

We recorded multiple unit activity bilaterally from the locus coeruleus during kindling of the amygdala in the unrestrained rats. We recorded the amplified signal from the LC and the amygdala and the output of a window discriminator (mentor-N750) adjusted to trigger only on unit activity that is at least two times the level of the baseline noise. The discriminator allowed us to screen out large artifactual signals, those that are often due to movement.

Multiple unit activity increases following the afterdischarge for a short time and decreases before the next AD in the amygdala. We often observed a very short period of inhibition during the amygdala AD.

These results are consistent with the idea that LC activation produced by AD's during amygdala kindling may provide a negative feedback that contributes to an inhibition of the seizure spread. This could be the basis for the longer time normally spent in the early stages of the kindling process. (Supported by Grant DHHS SO6 RR 08139-11 NIH)

385.7 SEIZURE THRESHOLD DETERMINATION AND STABILITY IN AMYGDALOID-KINDLED RATS. G. Cloutier*, W.W. Pugh. North Carolina Foundation for Mental Health Research, Raleigh, NC 27611 and Medical Division, Burroughs Wellcome Co., Research Triangle Park, NC 27709

Adult male Long Evans rats were implanted with bipolar stimulating/recording electrodes in the unilateral amygdala. Standard kindling procedures were employed to develop the amygdaloid-kindled model of complex partial epilepsy. An animal was considered fully kindled after three consecutive generalized seizures were elicited. Behavioral (clinical stages 0-5, post-ictal depression) and electrophysiological (after-discharge duration, post-ictal spiking) parameters were examined. An additional measure, seizure threshold, was also investigated. Kindled animals were tested for seizure threshold at intervals ranging from five minutes to five days. Electrical stimulation (60 Hz, 1 sec) was delivered beginning with low currents and increasing the stimulation current every five minutes until a generalized seizure (after duration >30 sec, behavioral state >2) was evoked. While seizure threshold determination is not usually defined in conventional terms of ictal (after-discharge, behvioral state) or post-ictal (post-ictal spiking, post-ictal depression) events, it also remained stable in the fully kindled animal. However, seizure threshold is modified or changed depending upon several variables. The first and most consistent is the interval period between generalized seizures. With very short intervals (5 minutes) the animal is refractory to a generalized seizure at currents up to two times normal threshold. Extending the seizure intervals decreased seizure threshold relative to increasing intervals. The greatest change in seizure threshold vs. seizure interval occurred in the first twenty-four hours. While additional decreases in threshold with increasing seizure intervals in excess of 24 hours were occasionally observed, the average animal exhibited stable seizure threshold over time with seizure intervals of at least 24 hours. A second determinant of seizure threshold was the overall state of health of the animal. Chronic pathology such as peritonitis or osteomyelitis decreased seizure threshold in direct correlation to the progression of the disease. Drugs were a third modifier of seizure threshold. Influences on seizure threshold, ictus and post-ictus by opiate agonists and antagonists are currently under investigation in this epilepsy model.

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385.8 GENETICALLY EPILEPSY PRONE RATS SHOW A DEFICIT AND DEVELOPMENTAL DECLINE IN MEDIAL GENICULATE BENZODIAZEPINE RECEPTORS. J.E. Franck, D.L. Hjeresen, D.G. Baskin, P.C. Jobe and P.A. Schwartzkroin. Depts. of Neurological Surgery and Psychology, Univ. Washington, Seattle, WA 98195 and Division of Metabolism and Endocrinology, VA Medical Center, Seattle, WA 98108.

The genetically epilepsy prone rat (GEPR), type 3, shows an abnormally high seizure susceptibility to a variety of stimuli including high intensity sound. Recent evidence indicates that neural abnormalities are, in part, at the level of auditory nuclei. There appear to be alterations in the GABAergic neuron population in inferior colliculus, and abnormal increases in glucose utilization in response to high intensity sound in GEPR colliculus, medial geniculate and auditory cortex. Our results lend further support to the notion that auditory pathway abnormalities in these animals underlie their seizure susceptibility, and indicate that these deficiencies operate at the level of receptors mediating inhibitory function.

We have examined the GABA receptor-chloride ionophore-benzodiazepine receptor complex in the medial geniculate of 24 and 31 day old unstimulated GEPRs and normal age-matched Sprague Dawley rats using quantitative autoradiogaphy of $^{3}$H-Flunitrazepam (FLU). Other work by us indicates that audiogenic seizure susceptibility does not develop in the GEPRs until 27-30 days of age. We wished to determine if differences in inhibitory receptors paralleled this behavioral phenomenon. Mounted tissue sections were incubated in increasing concentrations of $^{3}$H-FLU with or without unlabelled diazepam according to accepted neurochemical procedures. The slides were apposed to tritium sensitive film with brain mash standards containing various known concentrations of tritium. The film was exposed for two weeks, and computer-assisted densitometric analysis was performed on medial geniculate.

We found that 31 day old GEPRs have lower $^{3}$H-FLU binding in the medial geniculate than do age matched controls. This difference was not only seen quantitatively, but appeared quite dramatic on visual inspection. Scatchard conversion of the densitometric data demonstrated that both affinity and receptor number are lower in the epileptic mutants at this age. When 24 day GEPRs were examined, they also showed a lower receptor affinity, but the receptor number was comparable to that seen in normal rats. These data suggest that the GABA receptor complex in GEPR mutants is abnormal in at least the thalamic component of the auditory system. Furthermore, the pathology appears to be progressive and may parallel the development of audiogenic seizure susceptibility in these animals.

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385.9 ALTERATIONS OF TRH RECEPTORS IN BRAINS OF AMYGDALOID KINDLED RATS. S. Kajita*, M. Okamoto, K. Akiyama*, N. Ogawa† and M. Sato. Depts. of Neuropsychiatry, and †Neurochemistry (Institute for Neurobiology), Okayama University Medical School, Okayama 700, JAPAN.

Thyrotropin releasing hormone (TRH), a tripeptide, has been recently implicated in seizure disorders. We previously demonstrated that ICV administration of γ-butyrolactone-γ-carbonyl-L-histidyl-L-prolineamide citrate (DN 1417), a potent analogue of TRH, suppressed progression of amygdaloid (AM) kindling and AM kindled seizures in a dose dependent manner (Sato et al, Neurosci. Abst., 10:407, 1984). To investigate the proposed anticonvulsant action of TRH, both TRH-like immunoreactivity (TRH-LI) and specific TRH receptor binding were examined in rat brains kindled from left AM. Male Sprague Dawley rats received an electrical stimulation of sine wave (400 μA, 60 Hz, one second) once daily until stage 5 convulsion was achieved successively for five days. Subsequently, the animals were decapitated either 48 hours (48h.K) or 7 days (7d.K) after the last seizure, and the brains were dissected into 7 regions for the biochemical determinations which were performed as previously described (Ogawa et al, Peptides, 3:669-677, 1982). Animals with sham operation (SO) were used as control. In 48h.K group, TRH-LI was significantly elevated in the amygdala plus piriform cortex (SO, 91 ± 10; 48h.K, 303 ± 27***) and hippocampus (SO, 32 ± 7; 48h.K, 135 ± 19***), where values are expressed as mean ± S.E.M. (pg/mg protein) and *** means P<0.001 vs SO. In 7d.K group, however, TRH-LI in those brain areas did not increase significantly, indicating that the change in TRH-LI may result from a preceding seizure. By contrast, specific [3H]TRH binding at a single concentration of 12 nM increased significantly in the striatum of the two kindled groups (SO, 18.6 ± 2.4; 48h.K, 28.9 ± 2.0*; 7d.K, 36.1 ± 2.6**, mean ± S.E.M. of fmol/mg protein, *:P<0.05 and **:P<0.01 vs SO), but did not change significantly in other brain areas. Scatchard analyses of saturation isotherm of specific [3H]TRH binding indicate that both Kd and Bmax increased significantly (P<0.01) in the striatum of 7d.K as compared to SO. An inhibitory role of the striatum for generalization of epilepsy has been well documented. In light of this notion and together with our pharmacological evidence for TRH, the present study suggests that changes in the number of striatal TRH receptors may be associated with long-lasting seizure susceptibility of AM kindled rats.

385.10 SPONTANEOUS RHYTHMIC SYNCHRONOUS EVENTS IN HUMAN EPILEPTIC BRAIN AND NORMAL MONKEY HIPPOCAMPUS. M.M. Haglund and P.A. Schwartzkroin, Dept. of Neurological Surgery, Univ. of Washington, Seattle, WA 98195

The in vitro slice preparation has been used to study human epileptic tissue from both lateral temporal neocortex and medial temporal regions (including hippocampus). Spontaneous rhythmic synchronous events (SRSEs) arising primarily from medial regions of human temporal lobe (Science 223:709-712, 1984) have been further characterized. Hippocampus of normal monkey has also been studied in order to determine whether these events are indicative of tissue epileptogenicity, or whether they are manifestations of the circuitry of normal medial temporal lobe regions.

Our studies in human brain suggested the following characteristics of the SRSEs: 1) These events appear to be postsynaptic potentials. SRSEs often displayed multiple components, with the reversal potential of the primary component similar to that of a chloride-mediated IPSP. SRSEs were blocked by $10^{-5}$ M bicuculline methiodide in the bathing media. 2) Slices displaying SRSEs also had functional IPSPs. 3) Cell polarization did not block or alter the occurrence or frequency of SRSE activity. Orthodromic stimulation interposed between spontaneous events could reset the SRSE rhythm. 4) Two distinct cell populations participated in SRSE activity. The predominant cell type resembled the pyramidal cell of hippocampus; in these cells, SRSEs could be quite complex, but triggered only one or two action potentials. The other cell type was more similar to the hippocampal interneuron; spiking activity associated with the SRSEs occurred as burst discharge. This burst normally "led" the event in the pyramidal cell type.

Because normal control tissue is difficult to obtain from human brain, we examined the hippocampus of normal monkey in experiments similar to those carried out on human epileptic tissue. Spontaneous rhythmic synaptic events were recorded from the pyramidal cell region of hippocampus, but not from dentate granule cells or from pyramidal-like cells of lateral temporal neocortex. These SRSEs were identical in all respects to those recorded from the human epileptic brain. These findings suggest that SRSE activity does not reflect tissue epileptogenicity per se. However, given that synchronous activity is an essential feature of epilepsy, the existence of synchronous synaptic events in brain tissue which tends to be epileptogenic is particularly interesting. The circuitry and cell characteristics underlying generation of such rhythmic activity may be important determinants of the tendency of this tissue to produce epileptic discharge.

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385.11 CHRONIC ELECTRIC STIMULATION OF CENTROMEDIAN THALAMIC NUCLEI IN THE TREATMENT OF MEDICALLY UNCONTROLABLE SEIZURES. F.Velasco, M.Velasco*, C.Ogarrio*, J.Almanza* H.Ranqel*. Funcional Neurosurgical Service, Mexico City General Hospital, S.S., México (PO BOX 73032).

Ten patients suffering of clinical and EEG primary generalized incapacitating seizures, in spite of high dose and blood levels of anticonvulsant drugs, without evidence of severe or progressive brain damage proven by computarized axial tomography, underwent stereotactic implantation of electrodes, aiming both centromedian nuclei of the thalamus. Four contact, platinum-iridium, semiflexible electrodes, isolated but at the tips were used. Through these electrodes bipolar, biphasic, electrical stimulation (ES), was carried out, using pulses of 60-100 Hz., 0.1 msec duration, 200 to 500 microAmps, in trains of 1 min. every 5 min., alternating right and left sides, in daily sessions of 2 hrs. EEG recordings from scalp and depth electrodes were taken for periods of 4 hrs./day, starting 4 days prior the beginning of ES to 5 days after the onset of ES and weekly thereafter. Psychometric evaluation using Weis, MMPI, discrimination and memory tests, was performed before, 15 and 90 days after the onset of ES. Type and frequency of clinical seizures were monitored 24 hours a day for a minimun of 6 weeks and thereafter an ordinary record has been kept by the patient's family.
In 8 patients seizures were dramatically reduced or abolished since the begining of ES. In 3 of these patient anticonvulsive drugs were discontinued and in the other 5, reduced to half the initial dose. The other 2 patients showed significant improvement, although continued to have ocassional seizures and anticonvulsive medication was mantained. EEG seizures and interictal spikes were significantly reduced ($p<0.01$), particularly in the scalp leads. From the centromedian nuclei, interictal spikes and tonic discharges were recorded in the absence of clinical seizures. Psychometric tests performance was also significantly improved.

385.12 PROTECTIVE EFFECTS OF MOSSY FIBER LESIONS AGAINST KAINIC ACID-INDUCED SEIZURES AND NEURONAL DEGENERATION. Maxine M. Okazaki and J. Victor Nadler. Dept. Pharmacology, Duke Univ. Med. Ctr., Durham, NC 27710.

Intracerebroventricular (i.c.v.) administration of kainic acid (KA) produces a specific pattern of neuronal degeneration that probably results from the convulsant action of KA on the limbic system. Previous studies demonstrated that lesions of the hippocampal mossy fiber pathway prevent the destruction of CA3 pyramidal cells by i.c.v. administration of KA to anesthetized rats, whereas lesions of other pathways are ineffective. Mossy fiber lesions were suggested to prevent CA3 cell death by attenuating hippocampal seizures. The present study was undertaken to evaluate this hypothesis.

Male rats were subjected to either of two procedures: unilateral transection of the mossy fibers over a 4-mm length of the dorsal hippocampus or unilateral destruction of dentate granule cells with colchicine. Immediately afterward, rats were chronically implanted with a cannula in the lateral ventricle ipsilateral to the treated hippocampus and with bipolar EEG electrodes bilaterally in the hippocampal CA3 area and the basolateral amygdala. Unanesthetized animals were infused through the i.c.v. cannula with 1.41 nmol of KA. EEG was monitored for 4 hr after the infusion and the incidence and extent of neuronal degeneration were determined by silver impregnation coupled with cresyl violet.

Control animals developed repeated seizures which, after about 1 hr, became continuous, amounting to a status epilepticus. Ictal events appeared first either in the hippocampus ipsilateral to the cannula or simultaneously in both hippocampi. Mossy fiber transection protected 6 of 15 subjects from the development of status epilepticus, but destruction of mossy fibers with colchicine conferred no such protection. In many experimental rats, however, ictal events appeared in the contralateral (untreated) hippocampus prior to the ipsilateral (treated) hippocampus. Histological examination of control brains revealed massive neuronal degeneration in the ipsilateral CA3-CA4 area and much less damage to the contralateral CA3. Mossy fiber lesions always substantially reduced or abolished neuronal cell death in the ipsilateral hippocampus, even when the ipsilateral hippocampus experienced several hours of continuous seizure. Neurons in other brain regions were protected from degeneration only when the mossy fiber lesion prevented the development of status epilepticus.

These results suggest that the hippocampal mossy fibers constitute an important, though not an obligatory, link in the circuit responsible for the spread of KA seizures. Degeneration of CA3 pyramidal cells appears to be related not simply to the duration of seizure activity per se, but specifically to seizure activity conveyed by the mossy fibers. (Supported by NIH grant NS 17771.)

385.13 MORPHOLOGICAL EVIDENCE FOR INCREASED INHIBITION OF BASKET CELLS IN THE DENTATE GYRUS OF MONGOLIAN GERBILS. G.M. Peterson and C.E. Ribak. Department of Anatomy, University of California, Irvine, CA 92126.

More GABAergic neurons are found in the hippocampal formation of seizure-sensitive (SS) gerbils than in seizure-resistant (SR) gerbils. This difference has been found in all subregions of the hippocampal formation, but is most predominant in stratum granulosum of the dentate gyrus (Peterson et al., Brain Res., 1985). Furthermore, many mossy tufts (the en passent terminals of the dentate gyrus granule cell axons) are virtually depleted of vesicles in SS gerbils whereas almost all mossy tufts have a normal appearance in SR gerbils. To explain the mechanism by which increased numbers of inhibitory local circuit neurons can lead to increased excitation, we have proposed a model of disinhibition in which the basket cells inhibit one another, thereby effectively removing the granule cells from recurrent inhibition. To test this hypothesis with anatomical methods we have made immunocytochemical preparations to localize GAD and to identify GAD-immunoreactive basket neurons within or subjacent to stratum granulosum. These neurons were examined for GAD-immunoreactive axosomatic terminals. Plastic-embedded 1 um thick sections were used for the purpose of counting these GAD-immunoreactive terminals which form a pericellular nest around GAD-immunoreactive somata in SS but not in SR. GAD-immunoreactive neurons in the SS dentate gyrus generally had 2-3 times more GAD-immunoreactive terminals surrounding their somata then did the GAD-immunoreactive cells in the SR dentate gyrus. To correlate these light microscopic structures with their presumed ultrastructural counterparts, ultrathin sections were examined to identify the synaptic contacts made by the GAD-immunoreactive terminals adjacent to GAD-immunoreactive basket cells. These contacts were identified as the typical GABAergic axosomatic symmetric synapses in both SS and SR brains. These data show that GABAergic cells in the SS dentate gyrus have more GABAergic inhibitory axosomatic contacts and suggest that these basket cells are inhibited more than in the SR. This inhibition of basket cells may be sufficient to remove the tonic inhibition of granule cells and to cause a disinhibition which results in bursting discharge of granule cells. The loss of recurrent inhibition in this way may lead to seizure activity in the SS gerbil.

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386.1 SLOW FIELD POTENTIALS AND "BURSTLETS" DETERMINE BURST RATE IN PENICILLIN-PERFUSED HIPPOCAMPAL SLICES. J.H. Schneiderman. Dept. of Medicine, University of Toronto, Toronto, Can.

Field potential recordings in the distal apical dendrites of penicillin-perfused guinea pig hippocampal slices reveal two distinct phases. In 3.4 mM penicillin a slow field potential averaging 2.8 (range 1.2 to 4.3) seconds in duration may account for the entire interval between bursts in rapidly bursting preparations but in slower slices may be responsible for as little as 20% of the interval. The remainder of the interval consists of low amplitude, rhythmic potentials which we have called "burstlets" because they have the same configuration as the full bursts but are much smaller (75 to 250 uV compared to 1 to 5 mV). They are rarely seen with somatic recording electrodes at high concentrations but when the penicillin concentration is reduced to 0.85 or 0.425 mM their amplitude increases and they are present at the soma. In low-dose penicillin the frequency of full bursts is reduced in a dose-dependent fashion and their threshold is increased. The duration of the slow field potential is unchanged. The increase in interburst interval is due to a prolonged "burstlet" phase.

The peak of the slow field potential is negative in the apical dendrites and positive at the soma. It occurs prior to the peak of pyramidal cell afterhyperpolarization but is coincident with the peak of glial depolarizations. The entire slow potential is sensitive to changes in extracellular potassium concentration but the early portion is also sensitive to changes in extracellular chloride. The slow field potential tends to outlast the intracellularly recorded AHP as well as the glial depolarization. The later portion of the field potential often cannot be recorded at the soma but is negative in the apical dendrites. The depth profile of the slow potential is complex and suggests more than one site of generation.

In the dendrites the "burstlets" consist of a positive potential followed by a longer lasting negative phase. At the soma they are positive with superimposed negative spikes. The cellular correlate of these events is usually a depolarizing-hyperpolarizing sequence but occasionally at the same membrane potential either component may occur in isolation. They have the characteristics of postsynaptic potentials in that their frequency is not affected by membrane potential but their amplitudes are altered appropriately. The depolarizations were rarely of sufficient magnitude to reach threshold for firing action potentials. The "burstlets" appear to reflect synchronous synaptic activity which results in bursting of small numbers of neurons. The hyperpolarizing phase is accompanied by small glial depolarizations, suggesting that under these circumstances the IPSPs may be mediated by potassium.

386.2 STIB: AN IN VITRO MODEL OF KINDLING? A.C. Bragdon, D.M. Taylor*, L.C. Rigsbee*, J.O. McNamara and W.A. Wilson. Veterans Administration and Duke Univ. Medical Centers, Durham, NC 27705.

**Kindling** is an animal model of epilepsy in which repeated delivery of electrical stimulus trains to limbic structures produces a permanent, abnormal increase in neuronal excitability. The mechanisms underlying kindling are unknown. It would be advantageous to have an in vitro model of kindling with which to study these mechanisms.

**Stimulus train-induced bursting (STIB)** is an in vitro model of epileptogenesis in which repeated delivery of electrical stimulus trains to hippocampal area CA3 produces a long lasting, abnormal increase in neuronal excitability in hippocampal slices from naive rats. The similarities between kindling and STIB suggest STIB may be an appropriate model for studying the mechanisms of kindling.

To strengthen the relevance of this in vitro model to in vivo phenomenon, we sought to determine whether hippocampal slices from kindled animals behave as though they have already undergone STIB.

Male Sprague-Dawley rats received daily stimuli in the right lateral entorhinal cortex until 3-5 Class 5 seizures occurred. Implanted, unstimulated rats served as paired controls. Each kindled-control pair was studied one day following the last seizure of the kindled rat. Investigators preparing and studying the slices remained blinded to the rats' kindling status until after the data were analyzed. Two to three slices from the same hippocampus of each rat received our STIB protocol: Slices were studied in medium containing 3.3 mM K+, 1.3 mM Ca++, and 1.2 mM Mg++. Extracellular field potentials were recorded in s. pyramidale of CA3b. Electrical stimuli were delivered to s. radiatum of CA3. After stable responses were established, stimulus trains (60 Hz, 2 sec) at twice baseline intensity were delivered every 5 minutes until triggered and spontaneous bursts, and afterdischarges were produced or until 10 trains were delivered.

Slices from the four kindled animals achieved STIB distinctly faster than slices from the control animal. The number (mean ± SEM) of stimulus trains needed to induce each type of epileptiform activity was:

| | Triggered bursts | Spontaneous bursts | Afterdischarges |
|---|---|---|---|
| **Kindled** | 0.7 ± 0.3 | 4.6 ± 0.8 | 5.3 ± 1.4 |
| **Control** | 5.6 ± 1.2 | 8.4 ± 0.8 | 8.9 ± 0.9 |
| | p<0.0005 | p<0.005 | p<0.05 |

These findings raise the interesting possibility that the same mechanisms may underlie the development of both kindling and STIB.

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386.3 TRANSFER BETWEEN PICROTOXIN AND ELECTRICAL KINDLING IN THE RAT. D.P. Cain. Dept. of Psychology, U. of Western Ontario, London, Ontario, CANADA N6A 5C2.

The hypothesized participation of GABA systems in seizure development has been the subject of research in a number of laboratories but the exact nature of the participation of GABA systems in the kindling of convulsions is not clear. Therefore, kindling was examined using the intraperitoneal (i.p.) or intracerebral (i.c.) administration of repeated doses of picrotoxin, which blocks GABA-mediated chloride conductance. Transfer to electrical kindling was then tested in the same rats.

Four groups of male hooded rats received repeated, spaced (48hr) i.p. injections of picrotoxin in doses of 1.5, 2.0, 2.5 or 3.0 mg/kg and were videotaped for 1 hr, after which the tape was scored for convulsive behavior. Another group received repeated, spaced (48hr) injections of picrotoxin directly into the basolateral amygdala using a chemitrode, and the EEG was monitored for 1-6 hrs. Different rats in this group received a constant dose ranging from 1.0-10.0 ug. Another group that had been electrically kindled in the basolateral amygdala received repeated, spaced (48hr) i.p. injections of picrotoxin at 1.5 mg/kg and were videotaped and scored.

The i.p. picrotoxin groups showed a steep dose-response effect: the lowest dose (1.5 mg/kg) did not kindle at all, the middle doses (2.0 and 2.5 mg/kg) kindled generalized convulsions after a mean of 12 and 4 injections, respectively, and the highest dose (3.0 mg/kg) evoked a generalized convulsion after the first injection. The rats in the 2.0 and 2.5 mg/kg groups were then electrically kindled in the amygdala and kindled after a mean of 6.3 ADs, which was significantly faster than a saline control group (13.2 ADs; $p < .002$). The rats that had been electrically kindled prior to transfer to i.p. picrotoxin displayed a generalized convulsion after 12.75 injections; the control rats did not progress beyond stage 2 after 15 injections. In the i.c. group there was no clear evidence of a dose response effect, apparently because the moderate to high dose rats (2.5-10 ug) had considerable brain damage both near the cannula tip and in neuroanatomically related pathways, and AD could not be evoked.The low dose rats (1.0-2.0 ug) showed significantly faster electrical transfer kindling (6.3 ADs; $p < .02$) and considerably less brain damage.

The results show that repeated i.p. or i.c. picrotoxin can kindle convulsions and that kindling transfers strongly to electrical kindling. Thus, despite evidence of a lack of loss of GABA-mediated inhibition as causative in electrical kindling (Kalichman, 1982; Tuff et al., 1983), picrotoxin kindling transfers strongly to electrical kindling, as do many other types of chemical kindling. A common mechanism possibly located in reticular or brainstem areas may be partly responsible for the transfer effect.

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386.4 PHYSIOLOGICAL LEVELS OF ESTRADIOL AND KINDLED EPILEPTOGENESIS. Gary G. Buterbaugh. Department of Pharmacology and Toxicology, University of Maryland School of Pharmacy, Baltimore, MD 21201.

Forty adult, female Sprague-Dawley rats were ovariectomized and implanted with an amygdala bipolar electrode and a s.c. silastic capsule containing cholesterol or 10% estradiol ($E_2$) in cholesterol to deliver serum levels of 40-50 pg $E_2$/ml. Ten days later, daily amygdala stimulation (1 sec of 60 Hz, biphasic square-wave pulses of 1 msec duration) at twice afterdischarge (AD) threshold was continued until two stage V seizures. Rats with $E_2$ required significantly fewer AD's (11.5±0.6 vrs 14.9±0.7) and less AD sec (593±39 vrs 825±65) to kindle. The difference in AD's to kindle was uniform throughout acquisition; the difference in AD sec was limited to the stages of generalized seizures. These differences were limited to electrode tip placements in the basal-lateral or cortical amygdala nuclei. AD in the contralateral cortex appeared earlier during kindling of rats with $E_2$ and with cortical amygdala nucleus placements. Preliminary results indicate that the effect of $E_2$ on kindling rates is even more marked when rats are kindled by stimulation of the frontal cerebral cortex.

Following kindling, the treatments were reversed in some rats. After 10 stimulation-free days, an increased retention of postictal myoclonic jerks was found in $E_2$-replaced rats (4.8±1.3 vrs 1.3±0.5). The presence of $E_2$ during kindling and/or post-kindling was also associated with 2-5 sec EEG bursts accompanied by brief motor seizures which sometimes developed into a secondary stage V seizure. These post-kindling events were unrelated to electrode tip placement during kindling.

Fourteen days after kindling, rats were administered pentylenetetrazole (PTZ), 20 mg/kg i.p., 20 minutes before suprathreshold stimulation. PTZ increased the severity of the stage V convulsion but did not effect AD duration. Rats with $E_2$ showed a significant increase in myoclonic jerks compared to rats without $E_2$ regardless of the presence of $E_2$ during kindling.

Sixteen days after kindling, rats received 5 suprathreshold stimulations at 48 hr intervals. These stimulations resulted in an increasing number of myoclonic jerks, EEG bursts and secondary stage V seizures. Rats without $E_2$ showed no evidence of EEG bursts or secondary seizures.

The results suggest that the effect of $E_2$ on kindled seizure acquisition is related to mechanisms of seizure generalization and may depend upon the specific neuronal circuitry accessed by AD. The effects of $E_2$ on fully kindled seizures suggest that excitatory effects of $E_2$ interfere with, or suppress the acumulation of, postictal seizure inhibition. (Supported by PHS NS 20670)

386.5 STATUS EPILEPTICUS FACILITATED BY PILOCARPINE IN AMYGDALA KINDLED RATS. Hillary B. Michelson, David O. Keyser* and Gary G. Buterbaugh. Department of Pharmacology and Toxicology, University of Maryland School of Pharmacy, Baltimore, Maryland 21201.

Ten male, Sprague-Dawley rats, 90-100 days-old, were kindled by daily amygdala stimulation (1 sec of 60 Hz biphasic square-wave pulses, 1 msec duration) until two stage V convulsions. Three weeks later, rats (N=9) were administered pilocarpine, 20 mg/kg ip, 20 minutes before suprathreshold stimulation. The resulting stage V primary seizure was little effected by pilocarpine. However, the usual postictal depression was replaced by hyperactive exploratory behavior (N=6); within 30-90 sec, myoclonic jerks and EEG spikes appeared, followed by bursts of high-frequency EEG spiking and motor arousal which increased in frequency and severity until self-sustained continuous convulsions (status epilepticus; STEP) developed. In two rats, the primary seizure diminished and then merged into STEP. One rat developed STEP after a 7 minute delay. One rat was administered pilocarpine, 8 mg/kg iv, immediately after termination of the primary seizure; STEP a-peared within 45 sec. Stage II seizures during kindling were not effected by pilocarpine.

The STEP initially consisted of 10-12/sec, 600-1000 uV EEG discharge accompanied by stage III/IV convulsions. Brief (0.5-2 sec) periods of EEG suppression and motor arrest gradually dissappeared as stage I/II convulsions became continuous. In 3 rats, the STEP was allowed to continue for 4-5 hours when it gradually terminated to severe postictal depression.

Scopolamine, 25 mg/kg ip, prevented the pilocarpine-facilitated STEP, but had little effect on fully developed STEP. Several anticonvulsant drugs were tested after 1-2 hrs of STEP. Combinations of two or more drugs were necessary in most cases to terminate the seizures; diazepam (20 mg/kg) + phenytoin (40-80 mg/kg) was the most effective combination.

Juvenile, male, Sprague-Dawley rats were kindled by hourly amygdala stimulation when 28 days-old. Pilocarpine failed to facilitate STEP when these rats were 42-48 days-old and when 60-65 days-old. However, two of these rats developed STEP when tested at the adult age of 70-80 days-old.

The results indicate that amygdala-kindled epileptogenesis is associated with a marked sensitivity to muscarinic receptor activation during the immediate postictal period. This receptor activation may overcome postictal inhibition to result in prolonged and self-sustained EEG and motor seizures, which may require the fully mature brain for development and which are paradoxically resistant to muscarinic receptor antagonism. Pilocarpine-facilitated status epilepticus should be a useful model for the study of the mechanisms of STEP, the importance of postnatal maturation to STEP and the effectiveness of anticonvulsant drugs to terminate STEP.
(Supported in part by PHS NS 20670)

386.6 TAURINE IN FOCAL EPILEPTOGENESIS. P. Ente*, G. T. Golden, R. G. Fariello,(SPON: C. Bianchi). Neurology and Research, VAMC Coatesville PA 19320 and Thomas Jeff. Med. College, Phil. PA 19107

Taurine (TAU), a sulfur-containing amino acid, is differentially distributed in the mammalian CNS with the highest concentrations found in the cerebellum, striatum, hypothalamus and pineal gland. Lack of a specific antagonist for TAU has hampered studies seeking evidence for a pathogenic role for TAU in focal epilepsy, by demonstrating that specific blockage of TAU causes epilepsy.

Recently, a novel antagonist TAG (6-aminoethyl-3-methyl-4H, 1,2,4-bennzothiadiazine-1, 1-dioxide) has been discovered. TAG antagonizes the actions of TAU in vitro and in vivo. In preliminary studies aimed at characterizing the specificity of TAG, we have observed that an aqueous solution of 50 mM/ml TAG applied to the cortex of urethane anesthetized Sprague-Dawley rats consistently induces transient epileptogenic interictal discharges. The TAG-induced epileptiform activity was suppressed by an equimolar solution of GABA but potentiated by topical equimolar TAU and unaffected by glycine and B-alanine. The present evidence suggests that the epileptogenic action of TAG is unrelated to its antagonism for TAU. Specific bloackage of TAU responses is seen with TAG concentrations of 200 uM, whereas at 400 uM, glycine and B-alanine responses are also antagonized. In our experiments, concentrations of 200-400 uM did not elicit epileptiform activity, thus the TAU block cannot be considered essential for the appearance of epileptic spikes. Only at TAG concentrations known to have non-specific effects, did epilepsy appear and become potentiated by TAU and blocked by GABA. Thus it appears that other factors besides the TAU antagonism are responsible for generating the paroxysmal discharges. Possibly TAG (at high concentrations) has GABA antagonistic properties, and this may be responsible for the observed epileptogenic effects.

In other experiments, we have studied the effects of TAG application on multi-unit activity of cortical neurons. No change in firing rate was observed with concentrations having specific TAU antagonistic effects. Changes in the firing pattern occurred only when much higher concentrations were used. In addition, TAG cortical superfusion at concentrations between 200 uM to 75 mM failed to break electrical caudate stimulation-induced cortical inhibition.

The present studies do not support current speculation of a role for TAU in cortical physiology, nor of an involvement of TAU in cortical epileptogenesis. We are presently looking at single unit responses of cells in a TAG-induced (50mM and below) epileptic interictal focus and studying the responses of single cells in the TAG focus to microiontophoretic application of GABA, Glycine, and B-alanine.

386.7 **STIMULUS TRAIN-INDUCED BURSTING (STIB) IN HIPPOCAMPAL AREA CA3 IS INHIBITED BY BACLOFEN.** **H.S. Swartzwelder, C.P. Sutch*, A.C. Bragdon and W.A. Wilson. VA and Duke University Medical Centers, Durham, NC 27705.**

**Baclofen (Lioresal) is used clinically to treat spasticity, but has received little attention as a potential antiepileptic agent. Within hippocampi rendered epileptic by various means, area CA3 is the focus from which spontaneous epileptiform activity originates. Ault and Nadler (1982) have shown that baclofen inhibits the synaptic output of CA3 pyramidal cells, apparently as an agonist at $GABA_B$ (bicuclline insensitive) receptors. To explore its antiepileptic potential, we tested baclofen's effect on Stimulus Train-Induced Bursting (STIB), a new model of epileptogenesis (Stasheff et al, Soc Neurosci Abstr 10: 549, 1984 and Brain Res, in press).**

**Transverse, 625 um hippocampal slices were prepared in standard fashion from 150-200 gm, male, Sprague-Dawley rats, and studied in physiological medium containing 3.3 mM K+, 1.8 mM Ca++ and 1.2 mM Mg++. Extracellular field potentials were recorded in s. pyramidale of CA3. Electrical stimuli (100-600 uA, 50 us) were delivered to s. radiatum of CA3. After stable responses to single stimuli were established, stimulus trains (2 sec, 60 Hz) were delivered every 5 minutes until triggered and spontaneous population bursting was elicited. Baclofen, dissolved in ACSF, was bath-applied to the slices at varying concentrations to determine its effect on evoked and spontaneous activity.**

**Baclofen was remarkably effective in suppressing epileptiform activity in a dose-dependent fashion at concentrations which did not alter normal evoked responses. At 0.1 uM, baclofen abolished spontaneous bursting (SB), while triggered bursts (TB) were unaffected. At 1 uM, baclofen restored TBs to the waveform of normal evoked potentials. Normal orthodromic population spikes (OPS) were minimally affected at 1 uM, but were generally suppressed at concentrations above 10 uM. $ED_{50}$s for suppression of SB, TB and OPS were 0.03, 0.3, and 3.0 uM, respectively.**

**Baclofen is remarkably effective in restoring normal activity to this epileptogenic neural network. Further experiments to explore the antiepileptic efficacy of this drug are warranted.**

**This research was supported by the Veterans Administration and NIH grant NS-17771.**

386.8 A QUANTITATIVE IN VITRO AUTORADIOGRAPHIC STUDY OF MU AND DELTA OPIOID BINDING IN THE HIPPOCAMPAL FORMATION OF KINDLED RATS. B.J. Crain, K.-J. Chang and J.O. McNamara. Depts. of Anatomy, Anesthesiology, Medicine (Neurology), Pathology and Pharmacology, Duke University Medical Center and the Epilepsy Research Center, VA Medical Center, Durham, NC 27710 and Dept. of Molecular Biology, Wellcome Research Laboratories, Research Triangle Park, NC 27713.

Recent studies have shown that opioid peptide levels are altered in hippocampal formation of kindled animals. We therefore studied the distributions of mu and delta opioid binding sites in hippocampal formation of kindled and control rats using quantitative in vitro autoradiography. Animals received daily stimulations of the amygdala until they experienced three Class 5 seizures. Paired control animals underwent implantation of electrodes but were not stimulated.

Mu binding sites, labeled with $^{125}$I-FK-33824, showed a highly organized laminar distribution. Twenty-four hours after the last kindled seizure, mu binding was significantly decreased by 26% in stratum pyramidale of CA1 and by 16% in stratum radiatum and stratum oriens of CA1 and stratum moleculare of the suprapyramidal blade of the dentate gyrus. No differences were seen between kindled and control animals at 7 or 28 days after the last kindled seizure.

Delta binding sites, labeled with $^{125}$I-D-ala$^2$-D-leu$^5$-enkephalin in the presence of the morphiceptin analog PL-032, also showed an organized laminar distribution. Twenty-four hours after the last kindled seizure, delta binding was decreased throughout hippocampal formation but was significantly decreased by 21% only in stratum moleculare of the suprapyramidal blade of the dentate gyrus. Seven days after the last kindled seizure, delta binding was significantly decreased by 10-20% throughout CA1, CA3, and the dentate gyrus. At 28 days after the last seizure, however, no differences were found between kindled and control animals.

Since the decreases in mu and delta opioid binding are transient, they are unlikely to be the molecular basis of the permanent kindling phenomenon. Rather, since iontophoretically applied opioids increase granule and pyramidal cell firing rates, these changes in opioid binding may represent protective adaptive responses to repeated seizures.

386.9 **POTASSIUM-INDUCED EPILEPTIFORM ACTIVITY IN HIPPOCAMPAL AREA CA3 VARIES MARKEDLY ALONG THE SEPTO-TEMPORAL AXIS.** W.A. Wilson, A.C. Bragdon and D.M. Taylor* (SPON:W. Anderson). Depts. of Pharmacology and Medicine (Neurology), Duke University and VA Medical Centers, Durham, NC 27705.

The hippocampal slice preparation is widely used to study the physiology and pharmacology of epilepsy. Most such studies implicitly treat the excitability of different slices as uniform, regardless of their sites of origin along the hippocampal septo-temporal axis. This is despite well documented septo-temporal variations in a number of anatomical and biochemical parameters. Having observed that temporal-end slices appeared more excitable in area $CA_3$ than did septal slices, we set out to address this question directly.

Male, 220-385 gram, Sprague-Dawley rats were decapitated and both hippocampi removed. Slices 625 um thick were prepared from each hippocampus, and placed in holding chambers containing medium (ACSF) continually bubbled with a 95% $O_2$ - 5% $CO_2$ gas mixture. ACSF composition in mM: KCl 3.3, $CaCl_2$ 1.8, $MgSO_4$ 1.2, NaCl 120, $NaHCO_3$ 25, $NaH_2PO_4$ 1.23, dextrose 10. As they were taken from the tissue chopper, slices were placed sequentially in individual comparments of the holding chamber in order to keep track of their original location along the septo-temporal axis. Slices were studied in "high $K^+$" ACSF identical to that above except that [KCl] was 7 mM. Extracellular recording electrodes were placed in s. pyramidale of $CA_3$b. After slices equilibrated in high $K^+$ ACSF for at least 15 minutes, spontaneous population bursts were counted over at least 5 minutes. Angle of slicing (perpendicular at the temporal end vs. perpendicular at the septal end), order of slicing (temporal to septal and vice versa), and order in which slices were studied were systematically varied to ensure that none of these factors was-responsible for the observed differences.

Burst frequency showed a marked departure from uniformity along the septo-temporal axis. Burstsper minute increased from 33.1±3.1 (mean ± SEM) in the most temporal slice to a maximum of 54.9±2.8 in the third slice from that end, then declined gradually to a minumum of 8.2±4.1 in slice 13, near the septal end.

These data are important for two reasons. First, they suggest that particular attention should be directed to the temporal (ventral) hippocampus in in vivo and in vitro studies of neuronal activity in epilepsy. (For technical reasons, often only dorsal hippocampus is studied in vivo.) Second, they demonstrate that slice location along the septo-temporal axis is an important confounding variable for which one must control when comparing results between slices.

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386.10 DEPOLARIZATION SHIFT AND AFTERDISCHARGE GENERATION IN PENICILLIN-TREATED IMMATURE HIPPOCAMPAL SLICES: THE ROLE OF NMDA RECEPTORS. R.J. Brady & J.W. Swann, Section for Developmental Neurophysiology, Wadsworth Ctr. for Labs & Research, NYS Department of Health, Albany, NY 12201

Our laboratory has previously examined penicillin's ability to produce epileptiform discharges in the CA3 region of hippocampal slices obtained from immature rats 9-19 days of age (Swann & Brady, Dev. Brain Res. 12 (1984), 243-254). It was demonstrated that this treatment resulted in spontaneously-generated and stimulus-evoked intracellular depolarization shifts (DS) with coincident extracellular epileptiform bursts. These events were of a longer duration and lower frequency than the epileptiform activity seen under identical conditions in hippocampal slices taken from mature rats. The immature tissue was also shown to possess a pronounced capacity to produce prolonged afterdischarges of up to 30 sec in duration. The present studies investigate the ability of N-methyl-D-aspartate (NMDA) receptor antagonists to suppress ictal-like activity in the CA3 region of penicillin-treated immature hippocampal slices. Bath application of the potent NMDA-receptor antagonists 2-amino-7-phosphonoheptanoate (APH) and ketamine (KET) block spontaneous burst and afterdischarge generation. To produce this effect, a concentration of 5 mM APH is necessary while only 10 µM KET is needed.

Ketamine, a dissociative anesthetic, is thought to be a non-competitive inhibitor of the NMDA response. At concentrations that have strong anticonvulsant-like actions, bath application of KET suppresses NMDA responses. Intracellular and extracellular recordings of the response of TTX ($10^{-6}$g/ml) treated immature hippocampal slices show that bath application of 10 µM KET reversibly blocks responses to iontophoretically applied NMDA but not quisqualate or kainate. Intracellular records show that KET application does not significantly alter the resting membrane potential or conductance of CA3 pyramidal cells. 10 µM KET blocks spontaneous penicillin-induced epileptiform activity; slightly lower KET concentrations do not completely abolish this activity, but instead eliminates afterdischarges, decreases the duration of the DS and epileptiform burst and increases the burst frequency. These latter effects transform the pattern of discharges to one closer to that seen in slices taken from mature rats. In the presence of 10 µM KET, orthodromic stimuli will not evoke afterdischarges but will instead elicit a DS and epileptiform burst much like that seen in a mature slice. Taken together these results suggest that the NMDA receptor is an integral part of the mechanism of afterdischarge generation and that differences in the NMDA receptor population could explain the differences seen between mature and immature hippocampus.

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386.11 LOCAL EXCITATORY INTERACTIONS AND EPILEPTOGENESIS IN THE CA3 REGION OF IMMATURE HIPPOCAMPUS. J.W. Swann, R.J. Brady, & K.L. Smith.* Sec. for Develop. Neurophysiol., Wadsworth Ctr. for Labs & Research, NYS Dept. of Health, Albany, NY 12201

Previous studies from this laboratory have shown CA3 pyramidal cells of 1 to 2 week old rat hippocampus to have a marked capacity to generate prolonged (20-30 sec) afterdischarges when exposed to GABA A antagonists. Extracellularly these afterdischarges ride on the envelope of a prolonged negative field. Current Source-Density analysis suggests the negative field is produced by an inward current, generated at the edge of the cell body layer in stratum oriens -- a region referred to as the infrapyramidal zone (IPZ). Coincident intracellular recordings show pyramidal cells undergo a prolonged depolarization during slow potential generation. Experiments reported here were undertaken in normal media in an attempt to determine the physiological origins of these events.

High gain extracellular DC recordings from the IPZ have revealed localized spontaneous negative fields which are 0.5-1 mV in amplitude. These events rise rapidly from baseline and are 100-500 msec in duration. The frequency of unit firing increases during their course. Intracellular recordings from immediately adjacent pyramidal cell bodies reveal large (4-10 mV) unitary epsps coincident with these spontaneous negative fields. The time course of the epsps follows closely that of the fields. Orthodromic stimulation of distant pyramidal cells recruit these epsps. At threshold stimulation strength they are variable in latency, and often fail to be elicited, which suggests they are at least disynaptically mediated. Higher stimulation strength results in an orthodromic epsp followed by a large biphasic ipsp. These latter events mask the underlying di or polysynaptic epsp although at these times the coincident slow field is summated and several mV in amplitude. With repetitive (2Hz X 10sec) stimulation, the negative field becomes greatly potentiated. Simultaneous intracellular recordings reveal first a hyperpolarization of individual pyramidal cells followed by a gradual transition to a depolarized state. Often upon cessation of stimulation, a self-sustained afterdischarge ensues.

Since the unitary epsps recorded are most likely a product of pyramidal cell to pyramidal cell interactions, we have begun to examine the effects of excitatory amino acid antagonists on these events and afterdischarge generation. NMDA receptor antagonists (e.g. kynurenic acid) appear to selectively decrease the size of the spontaneous slow fields and prevent afterdischarge generation. These data suggest that an unusual degree of excitatory interaction may exist between immature CA3 pyramidal cells and could be a major factor contribution to the pronounced capacity of this region to undergo ictal-like discharges.

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387.1 ALPHA ADRENOCEPTORS AND AUDIOGENIC SEIZURE ACTIVITY IN DBA MICE. C.E. Lints, C. Nyquist-Battie, and D. Stearn*. Depts. of Psychology and Biological Sciences, Northern Ill. U., DeKalb, IL 60115.

We have previously reported that, compared with the seizure resistant C57BL strain, the DBA midbrain contains a higher density of beta adrenoceptors at an age (19-23 days) when these mice are most susceptible to audiogenic seizures (AGS). At this same age pindolol, a potent beta antagonist, produced dose-dependent anticonvulsant effects suggesting a proconvulsant action for beta receptors in these animals.

The present experiments compared alpha adrenoceptor densities and affinities in like-aged (19-23 days) C57BL and DBA mice. In these equilibrium studies the alpha-1 and alpha-2 binding sites on washed membrane preparations were labelled with (3H)-WB4101 and (3H)-yohimbine, respectively. Non-specific binding was that determined in the presence of (-)-norepinephrine (NE). Total amount of ligand bound and $K_D$ were determined by Scatchard analysis, and strain comparisons were made using two-tailed t-tests. Protein was determined using the Biorad Protein Assay Kit. There were no significant strain differences in affinity, as measured by $K_D$, for any of the brain regions studied. However, at this age of peak AGS susceptibility, the DBA midbrain contained a higher density of alpha-1 and a lower density of alpha-2 adrenoceptors than that of the seizure resistant C57BL mouse.

The effects of tolazoline, a nonselective alpha antagonist, on AGS activity were evaluated in 30-day-old DBA mice to screen for the possibility that these alpha receptor abnormalities are related to AGS. At this age the mice are less susceptible to seizures and both pro- and anticonvulsant effects can be easily assessed. Tolazoline had a triphasic action, with both the lowest and highest doses producing anticonvulsant effects and the intermediate doses producing proconvulsant effects. At this same age prazocin, a selective alpha-1 antagonist, produced dose-dependent anticonvulsant effects suggesting that the alpha-1 receptors also have a proconvulsant action. Yohimbine, a selective alpha-2 antagonist, had proconvulsant activity at a low dose that could be associated with autoreceptor blockade; higher doses produced anticonvulsant effects possibly due to yohimbine's local anesthetic-like action.

Taken together with our previous report of a higher midbrain beta receptor density, these results are consistent with the hypothesis that the DBA midbrain contains a locus of noradrenergic hyperexcitability that may be involved in the initiation and/or modulation of AGS in these genetically seizure-prone animals. They also suggest that the adrenoceptor subtypes can be independently regulated and that more than one mechanism may be involved (i.e. synaptic transmitter level and/or genetic predetermination).

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387.2 ANTAGONISM OF STRYCHNINE SEIZURES BY INTRATHECAL AMINISTRATION OF AMINO ACIDS AND ANTICONVULSANTS. D.K. Boyd*, P.A. Boxer and R.J. Anderson. Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48105.

Spinal seizures induced by strychnine are among the most refractory to treatment with anticonvulsant drugs. Systemic administration of amino acids, even glycine, fail to antagonize these seizures, presumably due to their inability to cross the blood brain barrier. The purpose of the present study was to determine whether direct application of drugs to the spinal cord would prolong or prevent strychnine seizures. In order to administer compounds intrathecally, mice were anesthetized with diethyl ether, the articulation between the 4th and 5th lumbar vertebrae was exposed and the L5 spinous process removed. All drugs were administered intrathecally in 5 microliters, 2 to 4 hrs after recovery from the anesthesia. Since subcutaneous administration of strychnine is often fatal and the latency to seizure is highly variable, we chose to induce a strychnine seizure via an intrathecal injection. Without pretreatment, an intratechal injection of strychnine (10 mM) produced seizures characterized by tonic hindlimb extension with a latency of 40 $\pm$ 15 sec. The optimum delay in the onset of these seizures was seen when the animals received an intrathecal injection of glycine two minutes before the strychnine injection. There was a concentration-dependent increase in the time to seizure with glycine concentrations of 30 to 1000 mM. Pretreatment with the inhibitory amino acid agonists, taurine (100, 300 mM), B-alanine (300 mM) and threonine (300 mM), significantly prolonged the seizure latency. On the other hand GABA (100 mM), glutamate (100 mM), sarcosine (300 mM) and serine (300 mM) produced no significant effects. The anticonvulsant diazepam (1 mM) significantly prolonged the seizure latency, while valproic acid (10 mM) had no effect. These results demonstrate that high local concentrations of several amino acids and anticonvulsants in the spinal cord prolong the onset of strychnine-induced seizures. However, none of these drugs was able to prevent the eventual development of a seizure, indicating that they have a short duration of action relative to strychnine.

387.3 EFFECTS OF DAILY MAXIMAL ELECTROSHOCK INDUCED SEIZURES (MES) AND CHRONIC PENTYLENETETRAZOL (PTZ) TREATMENT ON ANION AND CATION TRANSPORT AND MUSCARINIC AND BENZODIAZEPINE (BZ) BINDING. H.S. White*, S.Y. Chow*, T. Honda*, D.R. Gehlert*, R.T. McCabe, J.K. Wamsley and D.M. Woodbury. Depts. of Physiol., Pharmacol. and Psych., Univ. of Utah, S.L.C., UT 84108.

The present investigation describes the effects of daily MES and daily sub-convulsive injections of PTZ on seizure susceptibility, cerebral cortical (CC) anion and cation transport processes, and regional binding of $^3$H-N-methyl scopolamine (NMS) and $^3$H-flunitrazepam (FLU). One group of 40 rats was given a daily MES stimulus (150 ma for 0.2 sec at 60 Hz) through corneal electrodes for 3 weeks while another group received a daily subconvulsive dose (35 mg/kg, i.p.) of PTZ for 3 weeks. Once weekly, both minimal and maximal electroshock seizure threshold (EST) was determined and the values compared to a control group whose EST was also determined weekly. At the end of 3 weeks, 95% of the MES group exhibited a maximal tonic-clonic seizure following a MES stimulus as compared with 60% at day zero. In addition, the duration of the extensor (E) phase of the tonic-clonic convulsion increased and the flexor (F) phase decreased. This resulted in a 67% increase in the E/F ratio. All of these findings indicate increased brain excitability. Daily MES produced a significant increase in the minimal EST and a significant decrease in the maximal EST. At the end of 3 weeks, those animals receiving a daily subconvulsive dose of PTZ exhibited extensive CNS stimulation that was manifested by prolonged (>30 min) and sustained myoclonic jerks. Daily MES and PTZ produced a marked gliosis in the CC as suggested by an increase in DNA. Preliminary results suggest that although the number of glial cells are increased, their ability to regulate the extracellular environment of the brain is impaired since intracellular sodium was increased and intracellular potassium was decreased.

Autoradiographic localization of muscarinic and BZ receptors on tissue slices of brain was determined using standard autoradiographic techniques. Muscarinic receptors labeled with $^3$H-NMS were significantly increased in the striatum (16%) and superior colliculus (20%) and significantly reduced in the substantia nigra (25%) by daily MES stimulation. These findings are in agreement with a decrease in maximal EST. BZ receptors labeled with $^3$H-FLU were increased in the caudate putamen (51%) and superficial and deep layers of the CC. These findings are consistent with an increase in minimal EST, since an increase in inhibitory receptors within these areas would tend to inhibit the initiation of a minimal seizure. In contrast to daily MES, daily injections of PTZ produced marked and significant reductions in BZ binding in the nucleus accumbens (47%) and in the molecular (53%) and granular (44%) layers of the cerebellum. These findings suggest that there is a decreased inhibitory output from the cerebellum, and are consistent with the marked and prolonged myoclonic jerks observed following chronic administration of PTZ. Scatchard analysis of saturation data are presently under investigation. These studies illustrate the utility of chronic PTZ and MES as two distinct neurophysiological and neurochemical models for the study of epilepsy.

387.4 THE ROLE OF GABA AGONISTS AND ANTAGONISTS IN SEIZURE MODULATION IN A GENETIC MODEL OF GENERALIZED EPILEPSY. R.J. Lee*, A. Depaulis, R.W. Olsen and P. Lomax. Department of Pharmacology, School of Medicine and the Brain Research Institute, University of California, Los Angeles, CA 90024.

Recent studies have demonstrated a decrease in [$^3$H]-flunitrazepam binding in the brain of the seizure sensitive (SS) strain of Mongolian gerbil as compared to the seizure resistant (SR) strain. This abnormality in benzodiazepine binding may reflect changes in GABAergic neurons which might be involved in the seizure diathesis in the SS strain. Additionally, an increase in GAD immunoreactivity was found in the hippocampus of SS gerbils. To investigate the involvement of GABAergic transmission in modulating seizures a GABA agonist, muscimol, and a GABA antagonist, picrotoxin, were injected into the substantia nigra (SN) or the hippocampus (H) of SS gerbils.

Adult gerbils of either sex with consistent seizure scores ranging from 2-5 (on a scale of 0-6) were used in all experiments. The animals were anesthetized with pentobarbital (65 mg/kg) and bilateral cannula guides were implanted stereotaxically into either the SN or H. A post-operative period of at least 7 days was allowed before any testing was carried out. Gerbils were arranged in a Latin square design and either control (sterile water) or different doses of either muscimol or picrotoxin were administered via a microinjection syringe connected by polyethylene tubing to an injection cannula. Following injections, gerbils were tested by the triple handling technique. Seizure incidence and severity were noted.

In the SN, muscimol (50 ng) was found to inhibit seizures completely, while muscimol (25 ng) decreased the incidence of seizures to 20% (controls demonstrated 100% incidence of seizure activity). Picrotoxin (0-80 ng) injected into the SN did not induce any significant changes in seizure activity. Hippocampal injections of muscimol (25 ng and 50 ng) did not produce any significant changes in the seizure parameters.

These pharmacological findings confirm previous biochemical observations that GABA transmission in the SN is involved in suppressing seizure activity in the SS gerbil. The results are also consistent with other models of epilepsy: ECS (Iadarola and Gale, Science 218:1237, 1982) and kindling (McNamara et al., Eur. J. Pharmacol. 86:485, 1983).

387.5 EFFECT OF CHRONIC SENSORIMOTOR SEIZURES ON GABA AND BENZODIAZEPINE RECEPTORS. E.M. Santori*, T. Der, and R.C. Collins. Neurology and Neurological Surgery, and the McDonnell Center for the Study of Higher Brain Function, Washington University School of Medicine, St. Louis, MO 63110.

Clinical observation and animal experiments have documented that recurrent focal neocortical seizures progressively increase in severity. To gain insight into the basic mechanisms mediating the expansion of focal seizures, we have chosen to investigate GABA and benzodiazepine receptor changes.

Male Sprague-Dawley rats were stereotaxically implanted with bipolar electrodes in the cortical forelimb sensorimotor overlap zone. Once daily, the rats were stimulated with a two second train of monophasic pulses (1 msec duration; 50 HZ). Control animals were implanted with electrodes and received similar handling, but were not stimulated.

At the time of sacrifice the rats had been subjected to a mean of 41 stimulations. AD duration increased from an initial mean of 12 seconds to 109 seconds. Initially, the seizure response primarily consisted of clonic and tonic forelimb movements. By the end of the study, animals commonly showed bilateral convulsions with rearing and balance loss.

Rats were sacrificed 24 hours after their last stimulation. Brains were processed for quantitative autoradiography of GABA and benzodiazepine receptors using 3H-muscimol (MUS) and 3H-flunitrazepam (FLU), respectively. To survey the entire brain for changes in receptor density the binding was assayed at near saturating concentrations of each ligand.

MUS and FLU specific binding were unchanged in the focus and seizure pathways.

| | FLU (fmole/mg) | | MUS (fmole/mg) | |
|---|---|---|---|---|
| | control | seizure | control | seizure |
| Focus | 287 | 279 | 520 | 550 |
| Caudate | 116 | 123 | 310 | 340 |
| Globus pallidus | 161 | 159 | 300 | 300 |
| Ventrobasal thalamus | 143 | 146 | 750 | 880 |
| Substantial nigra | 307 | 291 | 350 | 380 |

Visual survey of the chronic seizure forebrains failed to reveal any unilateral binding changes. In addition, no bilateral perturbations of amygdaloid or hippocampal ligand binding were detected. These data suggest that sensorimotor kinding does not alter the density of GABA or benzodiazepine receptors within seizure pathways.

## HISTORY OF THE BRAIN

388 HISTORY OF THE BRAIN. F.A. Brewer*, E.M.R. Lomax*, L.H. Marshall, Y.V. O'Neil*, and H.W. Magoun. Brain Research Institute, UCLA, Los Angeles, CA 90024.

The objectives of this exhibit of 42 posters are to educate neuroscientists in an awareness of their rich heritage and roots in the past, to remind them of the importance of original source materials, and to demonstrate the usefulness of oral history in preserving antecedent events. Section I describes how three leading American publications in the history of neuroanatomy and neurology came into existence. They are The Founders of Neurology, The Human Brain and Spinal Cord: A Historical Study Illustrated by Writings from Antiquity to the Twentieth Century, and Garrison's History of Neurology. Section II of the exhibit traces the history of the human brain of particular clinical interest with emphasis on handedness and brain function. The evolution of the human brain is shown with evidence of lateralization in lower life forms and in early cultures. The search for the neural substrates of speech and consciousness is depicted from the Renaissance through the nineteenth century. Sections III and IV utilize the fascination of regional reminiscences in exploring the development of an early school of neurology in Los Angeles in the setting of its initial medical schools and hospitals. That story leads to an account of human commissurotomy and the shared Nobel prize in physiology or medicine in 1981. A free brochure describing the exhibit is available.

At the request of the authors the Society for Neuroscience wishes to retract abstracts 115.10 and 115.11 appearing in the 1984 Abstract Volume.